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Mureidomycin A and Dihydropyrimidine Nucleosides

Exploring efficient multicomponent reactions

and chemo-enzymatic approaches

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VRIJE UNIVERSITEIT

Mureidomycin A and Dihydropyrimidine Nucleosides

Exploring efficient multicomponent reactions and chemo-enzymatic approaches

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad Doctor aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. L.M. Bouter,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de faculteit der Exacte Wetenschappen
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door

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geboren te Tilburg

promotor: prof.dr. M.B. Groen
copromotor: dr. R.V.A. Orru

aan mijn ouders

Table of Contents

List of Abbreviations

AICAR	5-aminoimidazole-4-carboxamide ribonucleoside
AMBA	2-Amino-3-methylaminobutanoic acid
ADP	adenosine diphosphate
AMP	adenosine monophosphate
ATP	adenosine triphosphate
BB	building block
BDV	borna disease virus
BH	benzoyl hydrazide
br	broad
BSA	bis(trimethylsilyl)acetamide
BVDU	(*E*)-5-(2-Bromovinyl)-2'-deoxyuridine
Cbz	benzoyloxycarbonyl
CC	column chromatography
CF	continuous flow
d	doublet
de	diastereomeric excess
DEE	diethyl ether
DHPM	dihydropyrimidine
DIPEA	diisopropylethylamine
DMAP	dimethylaminopyridine
DMP	Dess–Martin periodinane
DMSO	dimethyl sulfoxide
DSC	differential scanning calorimetry
EA	ethyl acetate
ee	enantiomeric excess
EI	electron impact
EICAR	5-ethynylimidazole-4-carboxamide ribonucleoside
ED_{50}	median effective dose
equiv.	equivalents
FICAR	5-fluoro imidazole-4-carboxamide ribonucleoside
FPV	influenza virus A
GC	gas chromatography
GMP	guanosine monophosphate
GTP	guanosine triphosphate
*Hb*HNL	*Hevea Brasiliensis* Hydroxy nitrile lyase
HBV	hepatitis B virus
HIV	human immunodeficiency virus
HCV	hepatitis C virus
HCN	hydrocyanic acid
HMCV	human cytomegalovirus
HMDS	hexamethyldisilazane
HMPA	hexamethylphosphoric acid
HNL	Hydroxynitrile lyase
HPLC	high-performance liquid chromatography

HRMS	high-resolution mass spectra
HSV	herpes simplex virus
HWE/aza-DA 4CR	Horner-Wadsworth-Emmons/aza-Diels Alder four component reaction
IC_{50}	concentration at which cell growth is inhibited 50 %
IMP	inosine monophosphate
IMPDH	inosine monophosphate dehydrogenase
IPCAR	methyl-iodo-1-β-D-ribofuranosylpyrazole-3-carboxylate
IPdR	5-Iodo-pyrimidin-2-one 2'-deoxyribose
IPN	infectious pancreatic necrosis
IR	infrared
KU	kilo unit
LD_{50}	concentration at which lethality is 50 %
m	medium
MCR	multi component reaction
MIC	minimal inhitory concentration
MRD	mureidomycin
MS	mass spectrum
MTBE	methyl *t*-butyl ether
MVV	maedi-visna virus
MW	microwave
NAD	nicotinamide adenine dinucleotide
NaOCl	sodium hypochlorite
NDV	newcastle disease virus
NMP	N-methyl-2-pyrrolidone
NMR	nuclear magnetic resonance
*Pa*HNL	*Prunus amygdalus* Hydroxy nitrile lyase
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
PE	petroleum ether
PG	protecting group
PsRV	pseudorabies virus
Rf	retardation factor
RVF	rift valley fever
RSV	respiratory syncital virus
Rt	retention time
s	singlet/strong
SAR	structure-activity relationship
spp	species
SVR	sustained viral response
t	triplet
TBS	*tert*-butyldimethyl silyl
TEMPO	tetramethylpiperidinyloxy free radical
THF	tetrahydrofuran
TLC	thin layer chromatography
TSAO	2',5'-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl-3'-spiro-5''-(4''amino-1''2''-oxathiole-2'',2''-dioxide)

UDP	uridine 5’-diphosphate
UPA	uridylpeptide antibiotic
VEE	venezuelan equine encephalitis
VV	vaccinia virus
w	weak
YFV	yellow fever virus
ZMP	AICAribotide
5-AZA-CdR	5-aza-2’-deoxycytidine
4CR	four-component reaction
3-DC	3-deazacytidine
3-DU	3-deazauridine

1

Mureidomycin A

a New Potential Antibiotic

Over the last twenty years the incidence of life-threatening infections has increased while the number of immuno compromised persons has also increased. As a consequence the resistance of bacterial strains to current antibiotics like penicillins and vancomycin has become a major clinical threat. In the late eighties a family of antibiotics, the mureidomycins, has been discovered which act as antibacterial agent via inhibition of MraY, an essential enzyme in the peptidoglycan synthesis of bacteria. The mureidomycins have been investigated, but a clear inhibition mechanism has not been found. In this chapter a short summary will be given of what is known about the mureidomycins and the synthesis of their analogues.

1.1 Introduction

Some seventy-five years ago antibiotics became available. When first discovered, antibiotics were thought to provide a miracle cure, and they literally were. Infections that were fatal before 1900 were tamed to mere inconveniences during the 20th century. However, the misuse, overprescription and abuse of antibiotics has allowed resistant strains of bacteria to develop and they once again threaten health and life.[1] The first example of resistance to an antibiotic was observed in the early 1940s. Right after the introduction of penicillin, it was found that the Minimal Inhitory concentrations (MICs) for *Staphylococcus aureus*, which can cause severe food poisoning, increased.[2] In the period of the 1980s and 1990s very potent broad-spectrum antibacterials were introduced. However, this led to concomitant increase in antibiotic resistance.[3] The resistance of bacteria, fungi and yeasts against antibiotics can be described with three types of mechanisms: [4]

a) Drug modification or inactivation

b) Target modification

c) Modified Cellular Uptake

The development of resistance against antibiotics is a cause of serious concern and the search for new antibacterial agents is a hot topic. The problem adherent to this is that these new antibiotic agents will show also resistance leading to the question: when does the next generation of resistance factor emerge after new antibacterial agents have been introduced?

1.2 Targets for antibacterial drugs

Almost all of the major antibiotics act against three primary processes in the pathogenic bacteria: protein biosynthesis, DNA replication and repair, and cell-wall biosynthesis (Figure 1.1).[5-7] Protein biosynthesis offers many steps where antibiotics could disrupt the machinery; examples of these antibiotics are macrolides, tetracyclines, aminoglycosides and oxazolidinones.[5] The only class of antibiotics in current use that blocks DNA replication and repair are the fluoroquinolones that exert their action by blocking the enzyme DNA gyrase and a cognate topoisomerase.[8] Antibiotics directed against cell-wall biosynthesis include the β-lactam group of penicillins and cephalosporins as well as the glycopeptide antibiotic class, of which two members, vancomycin and teicoplanin, are approved for clinical use in humans.[5] However, resistance has been reported against all of these antibiotics, which is a cause of concern. One of the possible solutions to overcome this problem is to find new biological targets *e.g.* in the cell-wall biosynthesis on which no antibiotic is currently acting.

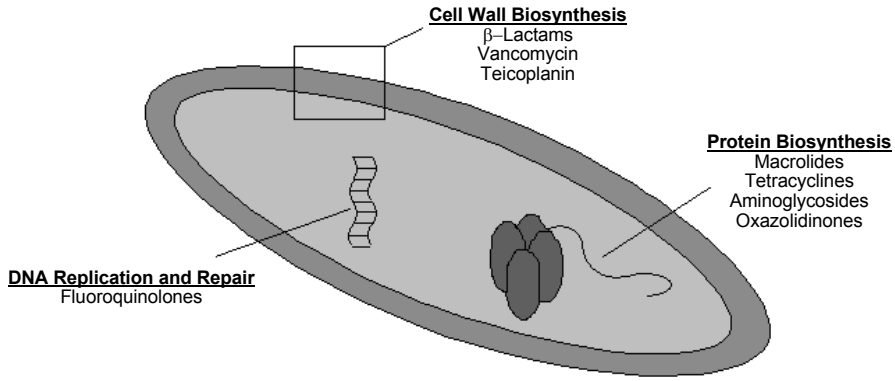


Figure 1.1 Bacterial targets of antibiotics

This thesis focuses on the mureidomycins (MRDs), and this new class of antibiotics has been shown to disrupt the bacterial cell wall synthesis. The biosynthesis of peptidoglycan is a complex three-stage process. The first stage involves synthesis of *N*-acetylmuramyl-pentapeptide by enzymes located in the cytoplasm, or at the inner surface of the cytoplasmic membrane. First UDP-*N*-acetylmuramic acid is formed from UDP-glycosamine. Then a pentapeptide is built up in a stepwise fashion by specific sequential actions of ATP-dependant ligases. (① in Figure 1.2). The second stage occurs on the inner surface of the cytoplasmic membrane where *N*-acetylmuramylpentapeptide is transferred from UDP to a lipid carrier, then modified to form a complete nascent peptidoglycan subunit. This compound then accepts GlcNAc from UDP-GlcNAc catalysed by transferase (③ in Figure 1.2). Transglycolase catalyses transfer of the peptidodisaccharide chain through the membrane followed by addition of the reducing end of a pre-existing peptidoglycan chain on the outside of the cell wall. The lipid pyrophosphate, which is released, is hydrolysed to the monophosphate by a lipid pyrophosphorylase, so it can re-enter the cycle (④ in Figure 1.2). In the last stage adjacent chains are cross-linked. This involves a transpeptidation reaction, with the cleavage of one peptide bond providing the energy needed to drive the formation of another peptide bond (⑤-⑥ in Figure 1.2).

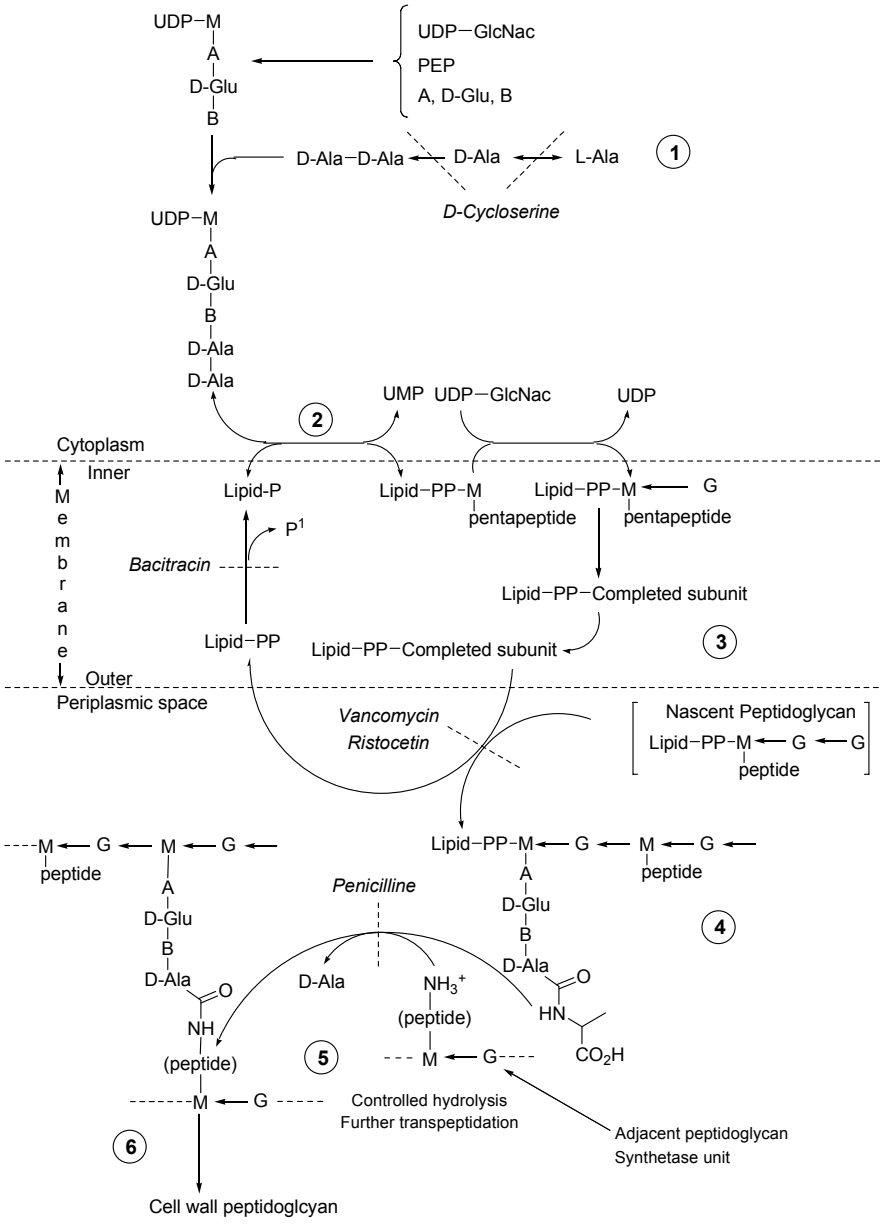


Figure 1.2 Biosynthesis of cell-wall of bacteria

1.3 MraY inhibitors

Phospho-MurNAC-pentapeptide translocase (MraY) is a catalyst in the peptidoglycan synthesis of bacteria (② in Figure 1.2 and Figure 1.3)[9] and catalyses the attachment of a phospho-lipid carrier to a pentapeptide. There are different inhibitors known that are active against MraY, and they can be classified into different groups (Figure 1.4).[10]

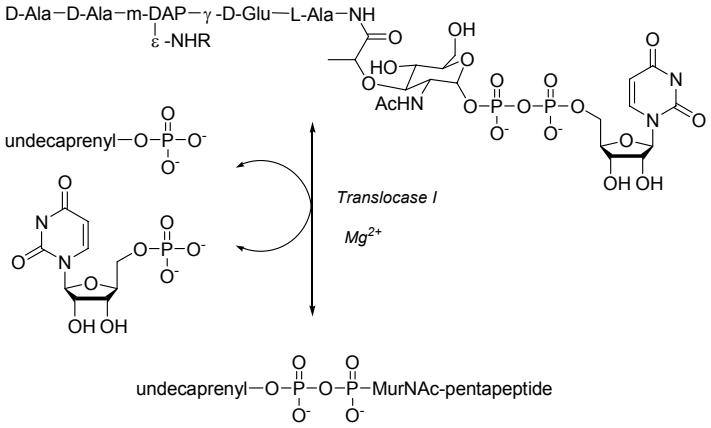


Figure 1.3 Catalysis by translocase I

Despite the number of inhibitors found active against MraY, it is not the target of any antibiotics currently used in the clinic. In general, these type of antibiotics block the synthesis of Lipid I, which is the product of the MraY enzyme reaction. However, the mechanism of inhibition appears to be different from one class to another. Most of these inhibitors have been tested in different assays, which makes it difficult to compare the reported 50 % inhibitory concentration (IC_{50}) values. Some of these inhibitors present antibacterial activities against Gram-positive bacteria (*e.g.* tunicamycins,[11] riburamycin[12] and amphomycin[13]), against *Mycobacterium spp.* (liposidomycins,[10] capuramycins[14]) and against *Pseudomonas spp.* (mureidomycins[15] and related compounds). Some are toxic and others are devoid of such drawbacks: tunicamycins are known to be highly toxic, while *e.g.* mureidomycins show good LD_{50} values when administrated to mice.[16]

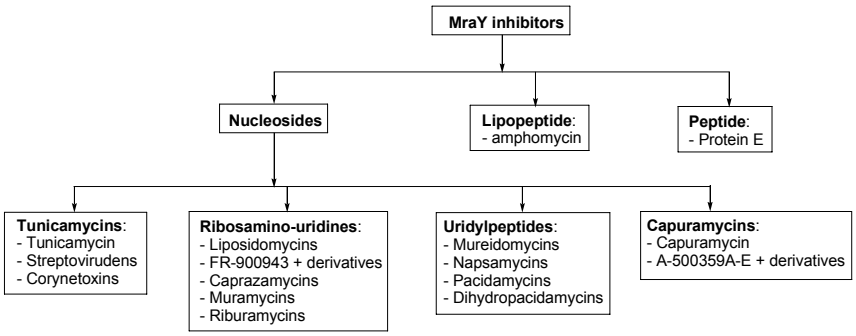
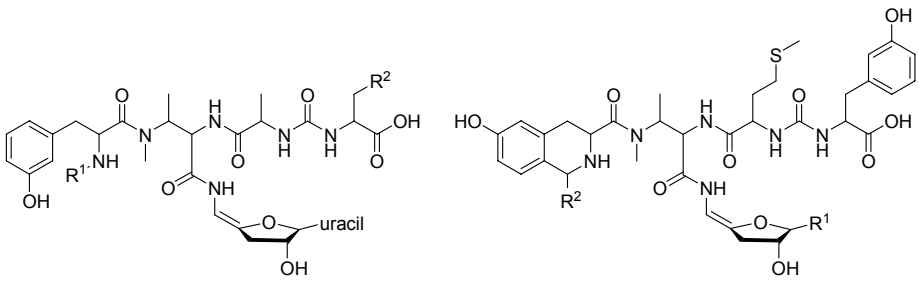


Figure 1.4 MraY inhibitors and classification

1.4 *Uridyl peptide antibiotics*

Mureidomycins, the molecules of interest in this thesis, belong to the class of uridyl peptide antibiotics (UPA) represented also by the pacidamycins[17] and napsamycins[18] (Figure 1.4 and Figure 1.5). UPA antibiotics share the same chemical template: a 3'-deoxyribose sugar is attached via an unusual enamide linkage to a peptide chain and linked by a glycosidic bond to a uracil or dihydrouracil. The still unexploited mode of action, their favourable toxicological and pharmacokinetic properties, and pharmacodynamic qualities against the highly refractory pathogen, *Pseudomonas aeruginosa*, makes this class of antibacterials especially attractive to investigate.[15c,17c]



Pacidamycin R^1= alanyl, H or phenyl
R^2= 3-indolyl, phenyl or *m*=OHC_6H_4

Napsamycin R^1= uracil or dihydrouracil
R^2= H or CH_3

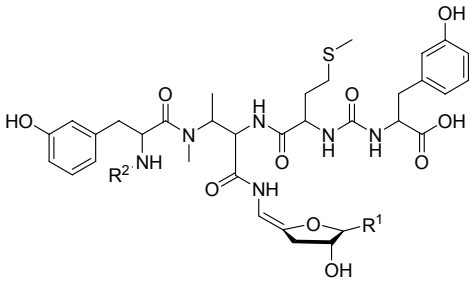
Figure 1.5 Uridyl-peptide antibiotics (UPAs)

However, the UPA class has two disadvantages which prohibited the introduction of a drug from this class. First, they have a limited spectrum; they are active against *P. aeruginosa*, an organism with high resistance to all current therapeutics, but show no

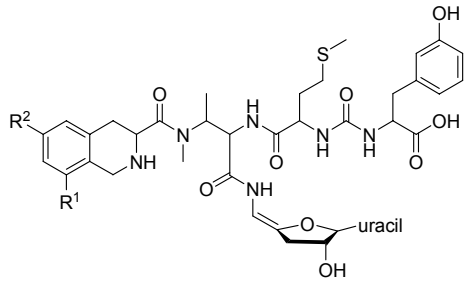
activity against any other significant human pathogen. Second, *P. aeruginosa* becomes resistant to these compounds at a frequency (10^{-5}-10^{-6}) that would restrict the usefulness of a UPA-based drug.[16c,17c]

1.5 Mureidomycins

The Mureidomycin family Mureidomycins A-D are isolated from *Streptomyces flavidovirens* SANK 60486[15] which show selective anti-pseudomonal activity, while not being toxic in mice. Mureidomycin E and F (=napsamycin with R^1 = uracil and R^2 = H) can be isolated from *Streptomyces flavidovirens* SANK 60486 or synthesised from mureidomycin A and formaldehyde via a Pincet-Spengler reaction.[19] They showed strong anti-pseudomonal activity, but less than mureidomycin A. This family of antibacterial agents, above all Mureidomycin A ([IC_{50}] is 0.05 µg/mL), exhibits a strong activity both *in vitro* and *in vivo* against strains of the difficult pathogen *Pseudomonas auruginosa*[20] and is as adequate as cefsoludin[21] and ceftazidime[21] (antibiotics currently in use) in a *in vivo* model of *P. aeruginosa* infection in a mouse.[22] It is active against isolated translocases from both *Escherichia coli* and *Staphylococcus aureus* although it lacks whole cell activity against these bacteria, indicating that these bacteria are still sensitive to MRDs. The resistance may be due to a permeability barrier to these agents. These interesting biological properties make the mureidomycin family, and especially mureidomycin A, an attractive synthetic target.[22]



Mureidomycin A: R^1 = uracil, R^2 = H
B: R^1 = dihydro-uracil, R^2 = H
C: R^1 = uracil, R^2 = glycol
D: R^1 = dihydro-uracil, R^2 = glycol



Mureidomycin E: R^1 = OH, R^2 = H
F: R^1 = H, R^2 = OH

Figure 1.6 The mureidomycin family

Mureidomycin A consist of a 3'-deoxyuridine nucleoside linked via an unusual enamide linkage to a modified peptide chain, which contains two *m*-tyrosine residues, one methionine residue, an unsymmetrical urea and one *N*-methyl-2,3-diaminobutyric acid residue. The closely related UPAs share the overall skeleton, but show small differences in the amino acid composition (Figure 1.7). All contain the pyrimidine nucleoside, the enamide linkage, a free amino terminus and the carboxy-terminal aromatic amino acid.

The molecular mechanism of inhibition of MraY by MRD A has been investigated. Brandish *et al.*[9a] have reported that MRD A is a slow binding inhibitor (K_i: 36 nM; K_i^*: 2 nM) of solubilised *E. coli* MraY. Slow-binding inhibition can be rationalised by the formation of an initial EI complex, followed by isomerisation to a more tightly binding EI^* complex, however the molecular basis for the EI to EI^* transition during slow-binding inhibition is unknown. In the next sub-sections the influence of the different parts of MRD regarding its activity will be discussed.

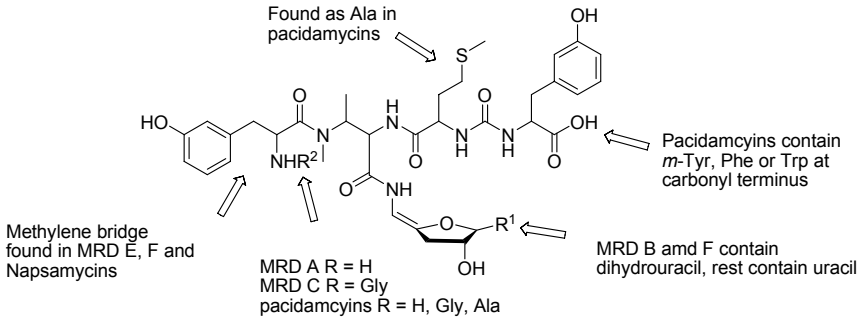
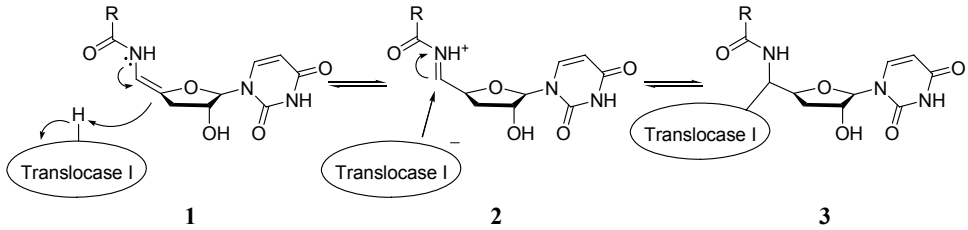


Figure 1.7 Relationships between the UPAs.

1.5.1 Role of enamide linkage on MraY inhibition

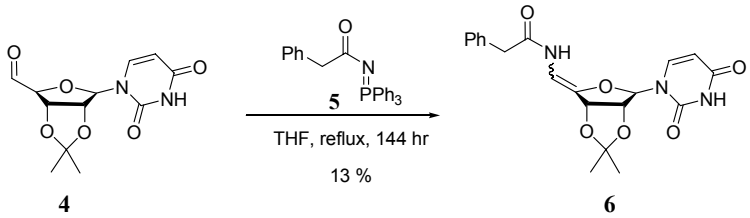
The presence of the enamide function is unusual and suggests that this feature plays an important role in the biological activity. Another unusual feature is the presence of a β-oxygen (the endocyclic oxygen of ribose) which might be expected to increase the reactivity of the enamide. There are two things that suggest that this enamide function could be important for biological activity of MRD A. First, enamides are able to generate *N*-acyliminium ion intermediates, which are readily attacked by a range of nucleophiles.[23] Secondly, the reactions catalysed by translocase I, are believed to occur via a two-step mechanism involving the attack of an active site nucleophile onto the β-phosphate of the substrate ADP-MurNAC-pentapeptide.[24] Gentle *et al.*[25] proposed the hypothesis for slow binding inhibition of translocase I by the enamide moiety at the enzyme active site to give *N*-acyliminium ion **2**, followed by attack of a nucleophilic amino acid side chain to give

covalent adduct **3**, as shown in Scheme 1.1. The pyrimidine nucleoside itself appears to be essential for the activity of MRD A, since all UPAs contain this moiety.[26]



Scheme 1.1 Hypothesis for mechanism of slow binding inhibition by Mureidomycin A

To study the relevance of the enamide linkage and to investigate the mechanistic hypothesis several enamide analogues have been synthesised. A simple analogue of MRD A (**6**) has been synthesised and revealed to be quite stable. Aldehyde **4** was coupled to an imino-phosphorane **5** via an Aza-Wittig reaction resulting in enamide **6** (Scheme 1.2).[25,27]



Scheme 1.2 Synthesis of uridine-based enamides

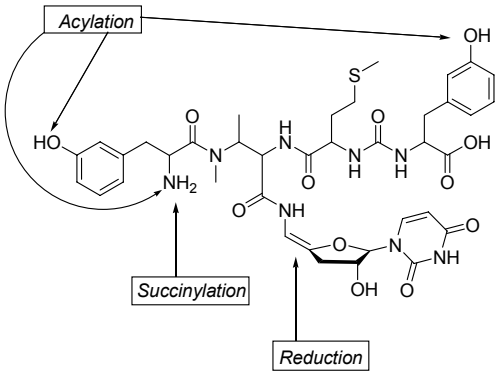
Enamide **6** and mureidomycin A show similar chemical inertness. The uracil base exerts an important influence on the properties of the enamide functionality, resulting in an increase in chemical stability. Apparently, a stereoelectronic effect of nitrogen substitution at the anomeric centre stabilises the enamide functional group. The lack of reactivity of mureidomycin A and **6** under a range of acidic reaction conditions coupled with the lack of inhibition shown by **6**, does not support the intermediacy of an *N*-acyliminium ion which is formed in the slow-binding inhibition of translocase I. However, it is possible that the enamide functional group in mureidomycin A is activated within the confines of the translocase I active site. Considering this together with the fact that dihydropacidamycin D, which lacks the enamide functionality, showed only slightly reduced activity against MraY,[28] implies that the enamide functional group is not primarily responsible for enzyme inhibition.

1.5.2 Solid-phase synthesis of MRD analogues

The solid phase synthesis of MRD analogues lacking the enamide group, the *N*-methyl group and the diaminobutyric acid *C*-4 methyl function has been accomplished by Bozzoli *et al.*[29] These analogues were not biologically active, implying that the methyl groups of the *N*-methyl diaminobutyric acid residue are important for biological activity.

1.5.3 Derivatisation of Mureidomycin A

Other traits that MRD A shares with closely related families are the free amino terminus and the carboxy-terminal in the aromatic amino acid. Gentle *et al*. investigated the importance of these functional groups for the inhibition of translocase I.[30] Mureidomycin A contains several functional groups that can be derivatised chemically (Figure 1.8). The activity shown by chemical derivatives of Mureidomycin A suggest that the amino terminus and the phenolic hydroxyl groups form significant binding interactions with the enzyme active site, although no single functional group was found to be essential for inhibition.[30]



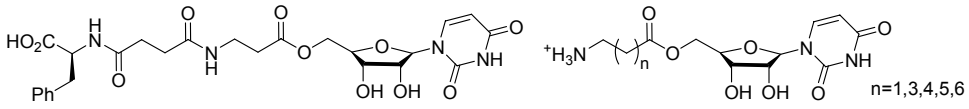
Reaction	Product	IC_{50} (μM)
Control	MRD A	<0.1
Succinylation	N-succinyl	7.0
Acetylation	Diacetyl	56/132
	Triacetyl	>100

Figure 1.8 Derivatisation of mureidomycin A

1.5.4 C- and N-terminus

The influence of the carboxy-terminal in the aromatic amino acid was studied by synthesis and testing of a *C*-terminus analogue **7** (Figure 1.9). Modest inhibition of this analogue showed that there is some binding interaction for the *C*-terminal part of MRD A, but that it is not strong by itself.[30] In order to further assess the role of the free amino terminus on the biological activity, a series of 5'-aminoacyluridine derivatives were synthesised by Gentle *et al*., containing a variable alkyl spacer (Figure 1.9). The results gave a surprising pattern of inhibition: only the 3-aminopropionyl (**8**) and 7-aminoheptanoyl (**11**) derivatives showed effective enzyme inhibition, but none was

observed for the 5-aminopentanoyl and 6-aminohexanoyl analogues, suggesting that there are different explanations for the inhibition observed for **8** and **11**.



7 **8-12**

Figure 1.9 *C*- and *N*-terminus analogues

It is known that MraY requires Mg^{2+} for activity. Gentle *et al*. suggested that MraY has a binding site as shown in Figure 1.10. The Mg^{2+} cofactor is bound relatively weakly by the enzyme, so it is reasonable that the inhibitors could bind to the active site in absence of Mg^{2+}. The vacant cation-binding site can now be occupied by the ammonium side chain. Gentle *et al.*[30] have suggested that **8** binds via a *syn*-ester conformation, while **11** binds via an *anti*-ester conformation (Figure 1.10). According to this model, the 5- and 6-aminoderivatives would be too long to bind in the *syn* conformation, while too short to bind in the *anti* conformation. Mureidomycin has a 7-carbon spacer between the uridine C-5' position and the amino terminus, consistent with the *anti*- conformation in Figure 1.10. This implies that the *N*-terminal part binds in the Mg^{2+} binding site, which brings the *m*-tyrosine residue close to the MurNAC binding site of the enzyme. The C-terminal *m*-tyrosine may bind instead the co-substrate undecaprenyl phosphate, which is also hydrophobic and negatively charged.

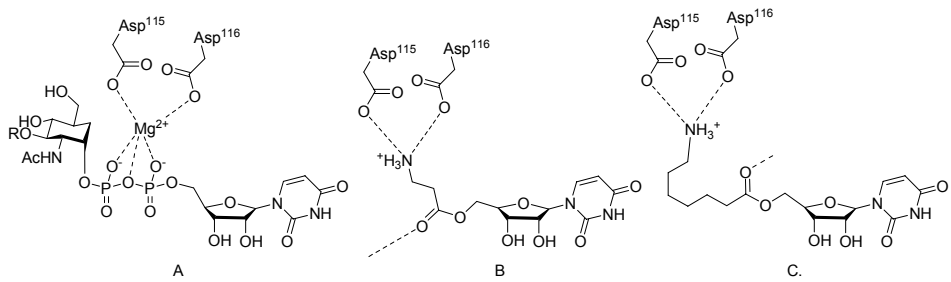
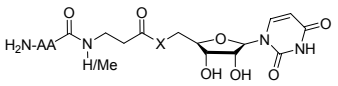


Figure 1.10 Proposed model for enzyme inhibition by aminoacyl-uridine models; A. Proposed role of Mg^{2+} cofactor and Asp-Asp pair; B. Proposed inhibition mode of 3'-aminopropionyl derivative; C. Proposed inhibition mode of 7'-aminoheptanoyl derivative.

In addition Howard and Bugg synthesised a series of 5'-uridinyl dipeptides, in which the dipeptide is linked to the uridine via either an ester or an amide (Figure 1.11).[31] The majority of the amide and ester analogues showed only 15-30 % inhibition at 2.35 mM concentration, however a few examples showed higher inhibition, especially **14C** (Figure 1.11).

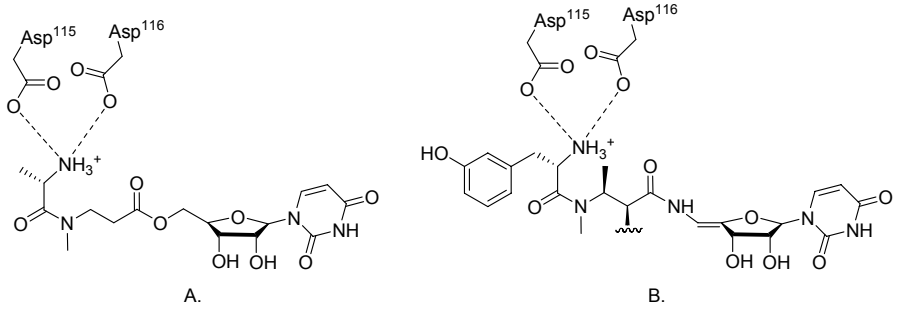


13 X=NH
14 X=O

Entry	AA	R	MraY inhibition of amide **13** (%)	MraY inhibition of ester **14** (%)
A	L-Tyr	H	48	24
B	β-Ala	Me	43	33
C	L-Ala	Me	14	97

Figure 1.11 The 5'-uridinyl dipeptides

Significantly lower activity was observed when either the *N*-methyl substituent or the ester linkage was absent, implying that these functionalities are both important for biological activity. This 5'-uridyl peptide was used to confirm the hypothesis that the amino terminus binds in place of the Mg^{2+} cofactor at the MraY active site. The enzyme assay used indicated that increasing concentration of Mg^{2+} reduces the potency of enzyme inhibition. In addition to the earlier proposed inhibition models, the model for **14A** is depicted in Figure 1.12A, where the amide bond has the *cis*-configuration and the ester function is flexible. The less reactive 5'-uridinyl dipeptides have a *trans*-amide conformation resulting in a less potent inhibitor. Mureidomycin A could also adopt a *cis*-amide conformation resulting from the enamide, as shown in Figure 1.12B. In this way their analogy in activity could be rationalised.



A. B.

Figure 1.12 A. Proposed model for enzyme inhibition by 5'-uridinyl dipeptides; B. Proposed binding mechanism of MRD to Mg^{2+} binding site of MraY, via cis-amide linkage.

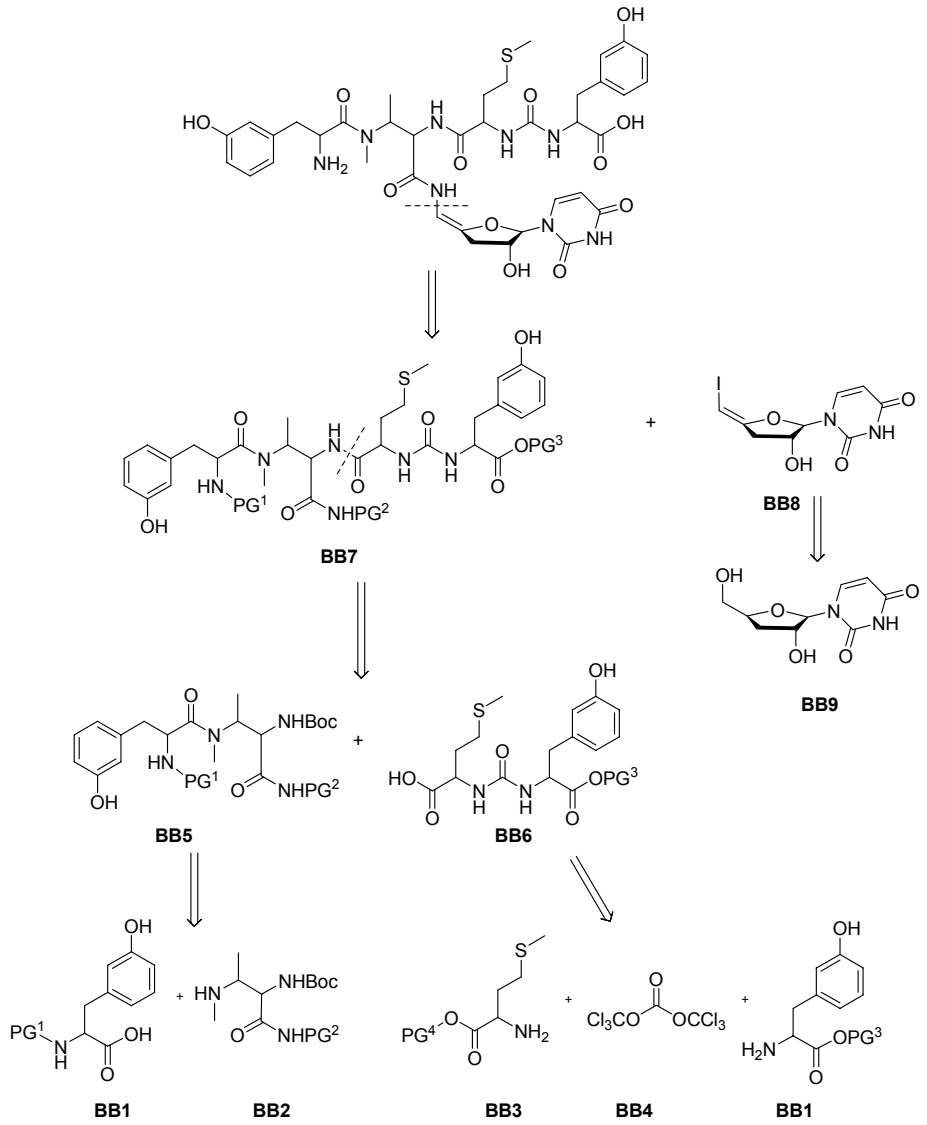
In conclusion, the slow binding inhibition by Mureidomycin A is due to several binding interactions from different parts of the molecule. More complex synthetic analogues will be needed to study more closely the tight binding to the enzyme active site.

1.6 Retrosynthetic analysis of Mureidomycin A

In the preceding section the different approaches to mureidomycin analogues and their biological activity have been discussed. In addition the total synthesis of dihydropacidamycin D has been reported.[28] Although a total synthesis of mureidomycin A is not the primary goal of the research described in this thesis, it is useful to draw a retrosynthetic analysis at this point. In order to keep the synthesis modular, we would like to introduce the enamide as late as possible in the synthesis of mureidomycin A.

The peptide backbone of mureidomycin A (**BB7**) consists of three building blocks (BB), a protected *m*-tyrosine (**BB1**), an amide analogue of AMBA (2-amino-3-methylaminobutanoic acid) (**BB2**) and an unsymmetrical urea (**BB6**). This urea can be divided again in three more building blocks, a *m*-tyrosine (**BB1**), a methionine (**BB3**) and triphosgene (**BB4**). The synthesis of unsymmetrical ureas from amino acids is known and is an uncomplicated synthesis,[32] apart from the synthesis of the *m*-tyrosine itself. The amide building block should be made from AMBA, and AMBA itself has been made before in the synthesis of dihydropacidamycin D.[28a] The synthesis of **BB 8** starting from **BB 9** can be envisioned via a vinyl tin nucleoside. Synthesis of an adenine analogue has been reported by Tanaka *et al*.[33] Building block **9** can be synthesised from uridine in four steps of which most of the reactions are protection and deprotection reactions.[34]

For the total synthesis of MRD A the protecting groups in the different coupling reactions have to be chosen wisely. Protecting group 1 (PG^1), as in Scheme 1.3, stays during the whole synthesis, and has to be removed very mildly, but has to withstand some peptide couplings. A Fmoc (9-fluorenylmethoxycarbonyl) protecting group seems to be a good choice. This group has to be removed under basic conditions, where enamides are stable. The second protecting group (PG^2) is an amide-protecting group. Removal of PG^2 should take place before, or during the iodide coupling reaction. A CBz group could be a good choice, because it can be reduced under conditions where the Fmoc group is not. The third protecting group (PG^3) is on the acid part of the unsymmetrical urea. This part has to be protected, because otherwise, it could couple with the AMBA-amide. This protecting group has to stay till the vinyl iodide coupling, and selectivity is not a problem, because it is the only protected acid. Good choices therefore could be a methyl or a benzyl ester.



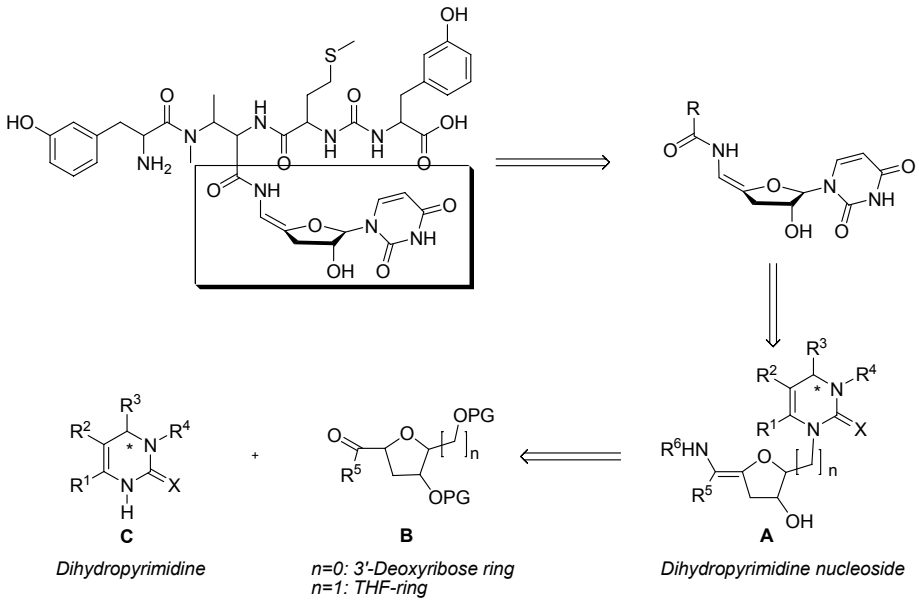
Scheme 1.3 Proposed retrosynthesis mureidomycin A

1.7 Goal

In this first chapter the mureidomycins, a class of natural antibiotics were discussed. These compounds contain a 3'-deoxy-ribose unit linked to uracil with a peptide backbone coupled via an unusual enamide bond (Scheme 1.4). This provides a challenging array of functionalities present in many other peptidyl nucleoside antibiotics as well. The methods

reported so far for the synthesis of such compounds most often involve a large number of steps and lack stereocontrol at some point. In addition only a few examples are known which do not use chiral starting materials. This limits the flexibility of the methods and makes them not suitable for the synthesis of a library of compounds needed for structure-activity relationship (SAR) studies. With regard to the synthesis of UPAs and its analogues, Boojamra *et al*. reported the synthesis of dihydropacidamycin D and a series of analogues, however, never with the unusual enamide linkage.[28a,35] In chapter 2 nucleoside analogues will be discussed. A large range of nucleoside analogues exists, but we focus on ribose sugars linked to monocyclic five- and six-membered heterocyclic bases that do not resemble one of the natural nucleobases in their core structure.

The combination of these two chapters leads to the goals of this thesis. The primary goal of the research is the synthesis of dihydropyrimidine nucleosides **A** (Scheme 1.4). The second goal is the total synthesis of mureidomycin A. In order to prepare **A**, an approach is followed that employs the versatility of a multi component reaction (MCR) in combination with the stereoselectivity of a chemo-enzymatic cascade reaction. A disconnection between the pyrimidine base and the 3'-deoxyribose moiety seems appropriate resulting in the 3'-deoxyribose moiety **B** and dihydropyrimidine (DHPM) **C**, which later could be combined (Scheme 1.4). The synthesis of 3'-deoxyribose moiety **B** is envisioned via a chemo-enzymatic cascade reaction and dihydropyrimidines **C** via a multi component approach.



Scheme 1.4 Goal of thesis

In *chapter 3* a new approach to dihydropyrimidines **C** will be discussed. The scope and limitations of a new four-component reaction have been explored resulting in dihydropyrimidines, phosphoramidates and triazinane diones. *Chapter 4* is an extension of chapter 3. Here the synthesis of thiazines using a similar four component approach and a subsequent rearrangement to the corresponding dihydropyrimidine-2-thiones is described. *Chapter 5* discusses a mild chemo-enzymatic oxidation-hydrocyanation protocol for the synthesis of cyanohydrins. These cyanohydrins are precursors for the 3'-deoxyribose moiety (**B**). In *chapter 6* the synthesis of such a 3'-deoxyribose moiety **B** will be discussed. Both a chemical and chemo-enzymatic route are described. *Chapter 7* summarises the synthetic studies towards mureidomycin A and dihydropyrimidine nucleosides **A**. The synthesis of the several building blocks of the peptide backbone will be discussed. In addition a model study is described about the coupling of dihydropyrimidines to ribose sugars.

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2

Biologically Active Nucleoside Analogues with 5- or 6- Membered Monocyclic Nucleobases

A large range of nucleoside analogues is known with a wide variety of therapeutic actions. In this chapter we discuss nucleoside analogues with a 5- or 6-membered monocyclic nucleobase, not having the core heterocyclic skeleton of a natural nucleobase and being attached to a 5-ring sugar (ribose) via a nitrogen atom.

2.1 Introduction

Nucleosides and nucleotides are an interesting class of compounds, which have gained their importance as biopharmaceuticals. Their biological activities range from antiviral, antibacterial, antifungal, antitumour, antitrypanosomal, herbicidal, insecticidal to immunostimulating.[1] This is not surprising, since nucleosides and nucleotides are precursors of DNA and RNA. Nucleosides and nucleotides consist of a sugar moiety attached to a heterocyclic base, and in nucleotides the 5'-OH is phosphorylated. The natural bases present in DNA and/or RNA have either the purine (**15**) or the pyrimidine (**18**) skeleton and are known as adenine (**16**), guanine (**17**), cytosine (**19**), thymine (**20**) and uracil (**21**). In natural nucleosides and nucleotides these are attached to 2'-deoxyribose (**22**) or ribose (**23**) (Figure 2.1).

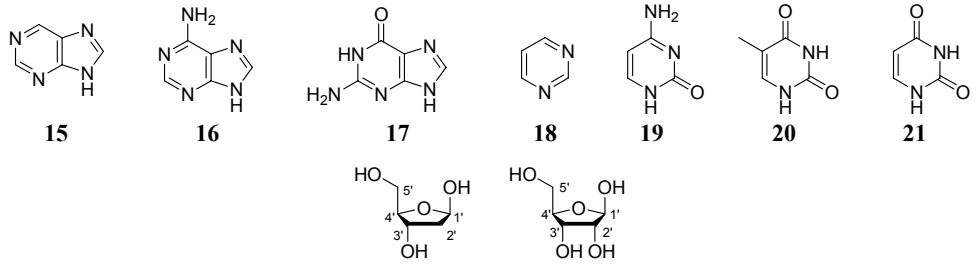
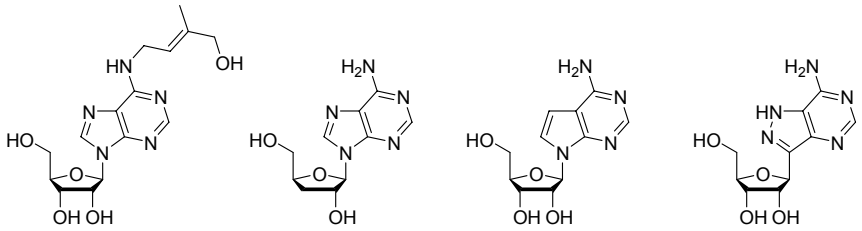


Figure 2.1 Bases and sugars in DNA and RNA

DNA and RNA contain the genetic information of the organism and in replication this genetic information is replicated also. Genes in living cells always contain DNA, while genes of viruses can consist of DNA or RNA. RNA viruses replicate through DNA intermediates. The genetic information goes from RNA to DNA rather than from DNA to RNA. For example, human immunodeficiency virus-1 (HIV-1), the cause of aids, uses this pathway and is also known as a retrovirus. Their RNA is transcribed to DNA by reverse transcriptase, an RNA-directed DNA polymerase. This implies that viral infections involve DNA and RNA.[2]

Nucleosides and nucleotides play also an important role in different metabolic processes in the human body and are intermediates in many biosyntheses. *E.g.* ATP is the major source of cellular energy and GTP is used as a source of energy in protein synthesis. Also nucleoside and nucleotide analogues, modified in either the base and/or the sugar moiety, are known to take part in metabolic processes.[3] For example, NAD^+, which is one of Nature's most important oxidising agents. In Figure 2.2 some examples are shown of other biologically active nucleoside analogues. *Cis*- and *trans*-N^6-(3-hydroxymethylbut-2-enyl)-

adenosine (**24**) are cytokinines, which are plant growth substances. Furthermore, cordycepin (3'-deoxyadenosine; **25**), tubercidin (**26**) and formycin (**27**) are all antibiotics.[3] Thus, nucleoside analogues are interesting compounds, either as regulators of, for example metabolic processes or for a range of pharmaceutical applications.

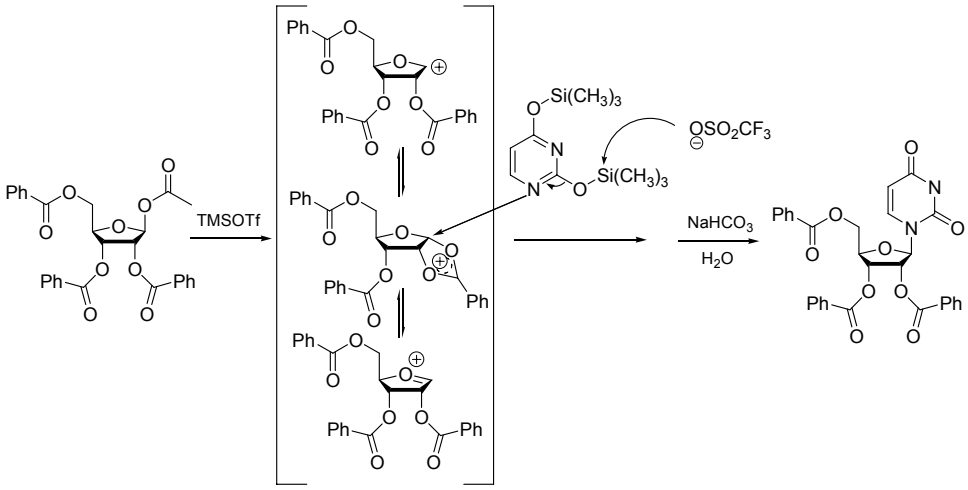


24 **25** **26** **27**

Figure 2.2 Several biologically active nucleoside analogues

Biologically active nucleoside analogues can differ from the natural-nucleosides in several ways. Either, the heterocyclic base can have substituents (**24**), the ribose is modified (**25**) or the base does not resemble the natural base in its core structure (**26-27**). In this chapter, we discuss nucleoside analogues with a 5- or 6-membered monocyclic base not having the core heterocyclic skeleton of a natural nucleobase and that is attached to a 5-ring sugar (ribose) via a nitrogen atom. We will focus only on biologically relevant nucleoside analogues. In section 2.2 the nucleoside analogues with a 5-membered nucleobase will be discussed. Here imidazole, imidazolinone, pyrazole, and triazole nucleosides are consecutively described. Next section (2.3) will address the nucleoside analogues with a 6-membered nucleobase, the pyridine, pyridinone, pyrazine, pyrimidinone and azapyrimidine nucleosides.

Mostly, the syntheses of the nucleoside analogues that are discussed in this chapter are based on the Silyl-Hilbert-Johnson method, better known as the Vorbrüggen condensation.[4] In this method, first the base is silylated using, for example hexamethyldisilazane (HMDS), and then a peracylated ribose together with a Lewis acid (TMSOTf) are added resulting in a nucleoside. In this way the β-anomer of the nucleoside is formed selectively (Scheme 2.1).[5] Often additional manipulations are necessary to obtain the desired nucleoside. Further synthetic details are only described when the approach significantly differs from the Vorbrüggen methodology.



Scheme 2.1 The Vorbrüggen condensation

2.2 *Nucleoside analogues with a 5-membered ring nucleobase*

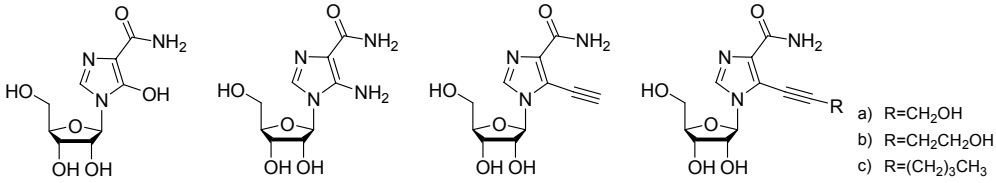
2.2.1 Imidazole nucleosides

In this class of nucleosides a large range of compounds has been synthesised and evaluated for their biological activity. In order of importance the imidazole nucleosides will be discussed starting with, bredinin/mizoribine, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) and 5-ethynylimidazole-4-carboxamide ribonucleoside (EICAR) followed by their analogue (Figure 2.3–2.8).

Mizoribine is an imidazole nucleoside isolated from *Eupenicillium brefeldianum* M-2166 and is structurally related to ribavirin (see section 2.2.4).[6] In 1984 mizoribine was clinically approved as immunosuppressant for renal transplantation, later for lupus nephritis (1990), rheumatoid arthritis (1992) and nephrotic syndrome (1995). Combination therapies with other immunosuppressive drugs like steroids,[7] azathioprine, methotrexate[8] or cyclosporin[9] are also in clinical use, because they exhibit excellent synergism. In addition mizoribine shows also antiviral activity, *e.g*. against influenza viruses type A and B,[10] bovine diarrhea virus (BDV),[11] hepatitis C virus (HCV),[12] and SARS.[13] The biological activities of mizoribine are resulting from the ability of its metabolites, the 5'-phosphate nucleotides, to inhibit inosine monophosphate dehydrogenase (IMPDH) and guanosine monophosphate (GMP) synthetase.[14] Mizoribine can be synthesised in different ways, either via enzymatic transformation of uridine,[15] or via two chemical pathways: Ueda *et al*. reported the synthesis via photolysis of the amine-analogue, resulting in a malonamide, which was reacted with ethylorthoformate resulting in mizoribine.[16] Shaw *et al*. reacted a protected

ribosylamine with malonic acid and subsequent amination and intramolecular cyclocondensation led to mizoribine.[17]

AICAR (**29** in Figure 2.3) is a good activator of AMP-dependant protein kinase (AMPK) when phosphorylated. Activation of AMPK is known to regulate glucose and fatty acid metabolism.[18] AICAR is phosphorylated by adenosine kinase to AICAR 5'-monophospate (ZMP), which mimics AMP and activates AMPK without altering the cellular levels of ATP, ADP or AMP.[19] ZMP is a key intermediate in purine biosynthesis and is usually immediately converted into inosine-5'-monophospate, the precursor of all purine compounds.[20] AICAR has shown apoptotic and anti-apoptotic effects, either induced by AMPK or not, *e.g.* in lung or prostate cancer cells.[21]



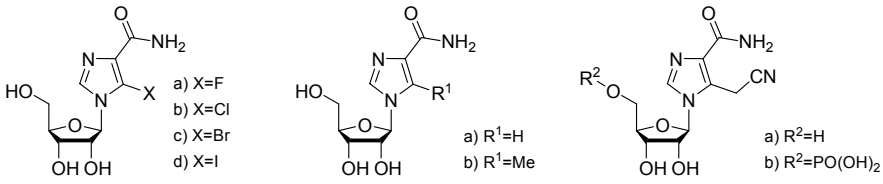
28 **29** **30** **31**

Figure 2.3 Mizoribine (**28**), AICAR (**29**), EICAR (**30**) and EICAR-analogues (**31**)

EICAR (**30** in Figure 2.3) is another imidazole nucleoside, which has been investigated extensively. EICAR shows *in vitro* activity against a range of viruses, such as, respiratory syncital virus (RSV), BDV, yellow fever virus (YFV), infectious pancreatic necrosis (IPN), measles-, pox-, toga- arena-, reo- orthomyxo- and paramyxoviruses.[22] The antiviral potency of EICAR is 10- to 100 fold greater than that of ribavirin.[23] Furthermore, EICAR exhibits antitumour activity and inhibits the growth of various tumour cells *in vitro*.[24,25] The mechanism of action is still unclear. Balzarini *et al*. showed that EICAR inhibits murine leukemia L1210 cells and human lymphocyte CEM cells.[23] This antitumour effect is caused by inhibition of IMP dehydrogenase. The 5'-monophosphate of EICAR was found to inhibit L1210 IMP dehydrogenase, too.[25] Several analogues of EICAR have been synthesised and evaluated for their antitumour activity, of which **31a-c** were the most profound.[25 a]

One of the other more interesting imidazole-4-carboxamide nucleosides is 5-fluoro imidazole-4-carboxamide ribonucleoside (FICAR (**32a**); Figure 2.4).[26] The antiviral and antimetabolic activities of this compound were compared to those of ribavirin and AICAR. The antiviral tests showed that ribavirin was generally most active, followed by FICAR and finally AICAR. The metabolic experiments revealed that ribavirin and FICAR are both potent inhibitors of cellular DNA and RNA synthesis, while AICAR did not show any inhibition; in fact it enhanced synthesis at a high dose. In addition, ribavirin and FICAR are both not cytotoxic.

Other 5-halogen-substituted (**32b-d**) and unsubstituted (**33a**) imidazole-4-carboxamide ribonucleosides were also tested and showed lower (**32b-d**) or comparable (**33a**) *in vitro* antiviral activity against herpes simplex virus 1 (HSV) and vaccinia virus (VV) than FICAR (**32a**).[27] The 5-methylimidazole carboxamide ribonucleoside **33b** showed a good antiviral activity against HSV, while no activity against other viruses was observed.[28]

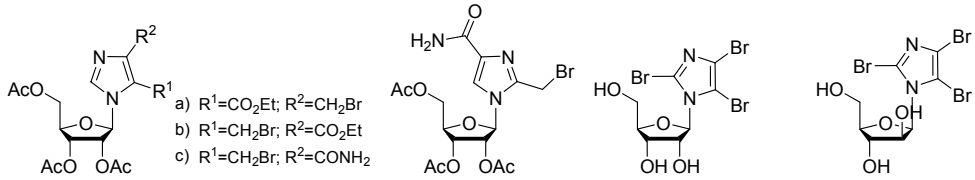


32 **33** **34**

Figure 2.4 Imidazole-4-carboxamide analogues

Robins *et al*. investigated the antiviral and antimicrobial activity of two 5-cyanomethylribofuranosyl imidazole-4-carboxamides **34a-b**.[29] Both show considerable *in vitro* activity against herpes viruses, while negligible activity against RNA viruses is observed and no antimicrobial activity. The 5'-monophosphate nucleoside **34b** shows also inhibition of IMP dehydrogenase, like ribavirin and mizoribine.

Other brominated imidazole nucleosides (**35** and **36**, Figure 2.5) have been reported to possess cytostatic activity against HeLa cells.[30] **35a-c** showed moderate ED_{50} values (10-50 μg/mL) while **36** had an ED_{50} of 5 μg/mL. In addition, Townsend *et al*. reported recently two tribrominated imidazole ribonucleosides with moderate human cytomegalovirus (HMCV) activity (**37** and **38**; Figure 2.5). However, toxicity of the compounds was higher than the respective IC_{50} value.[31]

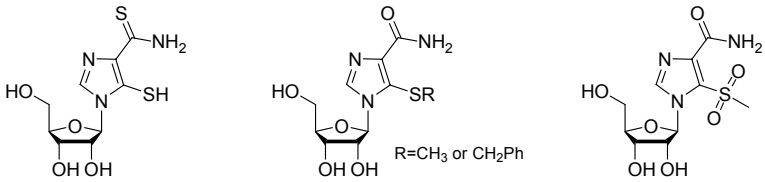


35 **36** **37** **38**

Figure 2.5 Bromo-imidazole nucleosides

Some thio-imidazole nucleosides (**39-41**) showed also activity against distinct viruses (Figure 2.6). Nucleoside **39** showed broad spectrum antifungal activity against *Candida Albicans*, *Trichophyton*, *Microsporum* and *Epidermophyton*.[27] Nucleosides **40** showed good antiviral activity against VV *in vitro*, however these activities were less than reported for

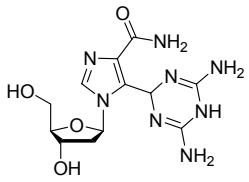
ribavirin, which is not a significant agent against VV.[32] Methylsulfonyl nucleoside **41** is *in vitro* active against several RNA viruses, like Rift Valley Fever (RVF) and Venezuelan Equine Encephalitis (VEE), while also good *in vivo* activity against RVF is observed.[32]



39 **40** **41**

Figure 2.6 Thio-imidazole nucleosides

Very recently, an imidazole nucleoside with a triazine substituent (**42**) has been synthesised and evaluated for its inhibitory activity against the West Nile virus NTPase/helicase. IC_{50} values of 3-10 μg/mL were reported.[33] Nucleoside **42** was synthesised via acidic work-up of 5-diethoxymethyl-imidazole-4-carboxylate riboside. Subsequent reaction with guanidine resulted in nucleoside **42**.[33]

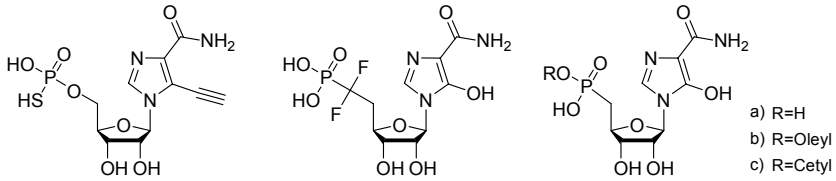


42

Figure 2.7 Triazine-imidazole nucleoside

Finally, 5'-monophosphate analogues of mizoribine and EICAR have been synthesised and evaluated for their antitumour or inhibitory properties of IMP dehydrogenase.[34] Nucleosides **43**, **44** and **45a** have been evaluated for their inhibitory properties of IMP dehydrogenase of human Type I and II and *Candida Albicans*. Although not nearly as good as the 5'-phosphates of ribavirin and mizoribine itself, the activities are reasonable and lie almost all in the range of EICAR. 5'-Monophospate nucleosides **45b** and **c** have been tested for their *in vivo* antitumour activity against ip- and sc- implanted tumours in mice and compared to mizoribine. The first experiment (ip-implanted tumour) showed that **45b** and **c** had no activity against P338 leukemia and Ehrlich carcinoma, while significant activity against MethA fibrosarcoma and Sarcoma180 was observed, in contrast mizoribine showed no activity against these three tumours. In the second experiment (sc-implanted tumour) only **45b** and mizoribine were

compared. Mizoribine did not show any activity against the tested tumours, while **45b** inhibited the growth of MethA fibrosarcoma and Ehrlich carcinoma.

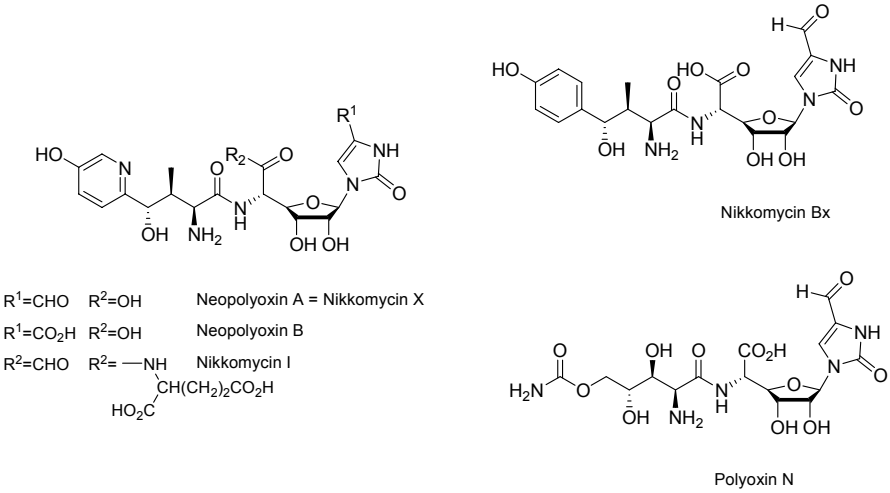


43 **44** **45**

Figure 2.8 5'-Phosphate imidazole nucleotides

2.2.2 *Imidazolinone nucleosides*

This class of compounds consists mainly of neopolyoxin A and B, polyoxin N and nikkomycins Bx, X and I (Figure 2.9). These are all peptidyl nucleosides that have been isolated from different *Streptomyces* strains.



R^1=CHO R^2=OH Neopolyoxin A = Nikkomycin X

R^1=CO_2H R^2=OH Neopolyoxin B

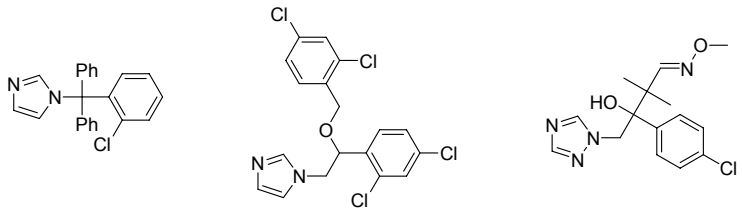
R^2=CHO R^2= —NH—CH(CH_2)$_2$$CO_2H$ Nikkomycin I

Figure 2.9 Different natural imidazolin-2-one nucleosides

Neopolyoxins A and B have been discovered by Isono *et al*. in 1980 and are isolated from *Streptomyces cacaoi* subsp. *Asoensis*.[35] Neopolyoxins are chitin synthetase inhibitors and are structurally related to the polyoxins, which have the same activity spectrum. Neopolyoxin A shows higher antifungal activity than neopolyoxin B and polyoxins, against a range of different fungi like *Pyricularia oryzae*, *Cochliobolus miyabeanus* and *Botrytis cinerea*.[35b,36] Polyoxin N has been isolated as a minor component from *Streptomyces piomogenus*.[37] As neopolyoxin A, it shows good antifungal activity against *e.g. Pyricularia oryzae*, *Cochliobolus miyabeanus*.[38]

Nikkomycins Bx, X and I are very closely related to the neopolyoxins, in fact neopolyoxin A is the same as nikkomycin X. However, the nikkomycins have been isolated from different strains, the *Streptomyces tendae* Tü901.[39] Like the neopolyoxins and the polyoxins, they are potent chitin synthetase inhibitors, because of their structural similarity to UDP-*N*-acetylglucosamine, the natural substrate of the enzyme. They show good fungicidal, insecticidal and acaricidal properties, while their toxicity against mammalian cells is low.[35,36,40] Combination therapy of nikkomycins X and Z with several azoles like clotrimazole (**46**), miconazole (**47**) or R3783 (**48**) (Figure 2.10) resulted in *in vitro* activity against *Candida Albicans*, which is normally poorly inhibited by these nikkomycins.[41]

The nikkomycins, X, I, Z and J show a clear difference in activity: while all are good chitin synthetase inhibitors, only dipeptides nikkomycin X and Z exhibit a good *in vivo* effect on *Mucor rouxii*. It is suggested that the uptake of the nikkomycins involves the transpeptidase reaction of the γ-glutamyl cycle, thus the dipeptides can be transported in this cycle, while the tripeptides are too bulky for uptake.[42]



46 **47** **48**

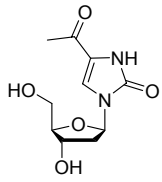
Figure 2.10 Azoles synergistic with nikkomycin X and Z

While nikkomycin Bx has not been studied as extensively as the other nikkomycins regarding its activity, much is known about the synthesis of this nucleoside. In 1987 Konig *et al*. reported the synthesis of nikkomycin Bx, its enantiomer and a *p*-methoxy analogue, nikkomycin X and its enantiomer. Only the natural occurring nikkomycins showed fungicidal activity, while the *p*-methoxy analogue showed activity against yeasts.[43] The nikkomycins were synthesized via coupling of the C-terminal nucleoside amino acid with the dipeptide resulting in the nikkomycins.

Rudd *et al*. reported in 1995 the biosynthesis of nikkomycin X from histidine in *Streptomyces tendae*.[44] L-Histidine is the source in *S. tendae* for the imidazolin-2-one moiety. In 2004 Tan *et al*. reported the enhanced production of nikkomycin X by over-expression of a non-ribosomal peptide synthetase in *Streptomyces ansochromogenes*, *SanO*. Twice as much nikkomycin X was produced compared to the wild type strain.[45] The biosynthesis of nikkomycins has been explored by Bormann *et al*, they report that 6 genes are involved in the synthesis; *nikU* and *nikV* are required for the synthesis of

hydroxypyridyl homothreonine, *nikP1*, *nikP2* and *nikS* for the assembly of nikkomycins and *nikT* for both pathways.[46]

Besides these peptidyl imidazolin-2-one nucleosides also one example of a non-peptidyl imidazolin-2-one nucleoside is known with biological activity. Imidine (**49**) is a 5-membered ring isomer of thymidine and its triphosphate competes with thymidine triphosphate for incorporation into DNA. The triphosphate is shown to be a potent inhibitor of HIV reverse transcriptase and causing premature termination of DNA synthesis and enhanced miscoding.[47]

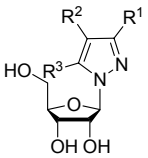


49

Figure 2.11 Imidine (**49**), non-peptidyl imidazolin-2-one nucleoside

2.2.3 Pyrazole nucleosides

In contrast to the triazole (see section 2.2.4) and imidazole nucleosides no pyrazole nucleoside is clinically in use. However, a series of pyrazole nucleosides (**50**) have interesting activities. The halogenated analogues of **50** seem most promising (Figure 2.12).[48-51]

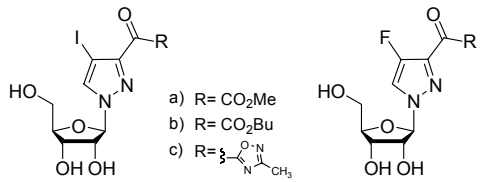


50

a) R^1= CH_2Br R^2= H R^3= CO_2Et
b) R^1= CH_2Br R^2= H R^3= $CONH_2$
c) R^1= $CONH_2$ R^2= H R^3= CH_2Br
d) R^1= CH_2I R^2= H R^3= CO_2Et
e) R^1= CH_2I R^2= H R^3= $CONH_2$
f) R^1= $CONH_2$ R^2= H R^3= CH_2I
g) R^1= $CH_2N(CH_2CH_2Cl)_2$ R^2= H R^3= $CONH_2$
h) R^1= CH_2Cl R^2= H R^3= CO_2Et
i) R^1= CH_2Cl R^2= H R^3= $CONH_2$
j) R^1= $CONH_2$ R^2= H R^3= CH_2Cl
k) R^1= $CONH_2$ R^2= NO_2 R^3= H
l) R^1= $CONH_2$ R^2= Br R^3= H
m) R^1= $CONH_2$ R^2= I R^3= H

Figure 2.12 Pyrazole nucleosides with cytostatic activity

Pyrazole nucleosides **50a-f** showed good cytostatic activity against HeLa cells,[48] while **50g-m** showed moderate activity (Figure 2.12).[48,49] In addition compound **50m** showed good antiproliferative activity against T-cell lines.[50] Several analogues of **50m** (**51-52**) showed comparable or even better antiproliferative activity against several lymphoblastic cell lines. While **50m** showed no effect on the proliferation of solid tumour-derived and embryonal cell lines, **51a-c** showed moderate (**51b**) and good (**51a** and **c**) activity (see also Figure 2.13).[51]



51 **52**

Figure 2.13 Iodo-pyrazole nucleosides and fluoro pyrazole nucleoside

Pyrazole nucleoside **51a** (IPCAR) has been tested for its cytotoxicity against proliferating and resting human PBL cells. Although non-cytotoxic to resting PBL-cells, IPCAR is cytotoxic to stimulated PBL-cells. Furthermore, IPCAR was found to promote the activity of ddI, an antiretroviral agent for the treatment of HIV-1. In conclusion, IPCAR seems to be a promising candidate for antitumour chemotherapy. Especially, the low toxicity for resting PBL cells and the promotion of the anti HIV-1 activity of ddI, makes this compound a potential candidate for the therapy of AIDS-associated neoplasias.[51] Although structurally related to ribavirin, La Colla *et al*. proposed that IPCAR does not inhibit IMPDH of the *de novo* purine biosynthesis. However, the exact mode of action remains unclear.[52] Finally, Coe *et al*. reported the anti-influenza activity of 4-fluoropyrazole nucleoside **52**, while no activity against several RNA and DNA viruses was observed.[53] This is in line with the other halogen substituted pyrazole nucleosides discussed above.

2.2.4 Triazole nucleosides

Triazole nucleosides consist of a sugar- and a triazole moiety. A triazole is a 5-membered ring that contains three nitrogen atoms. Depending on the position of the N-atoms in the ring, different types of triazoles are identified. Consequently, different types of triazole nucleosides are possible (Figure 2.14).

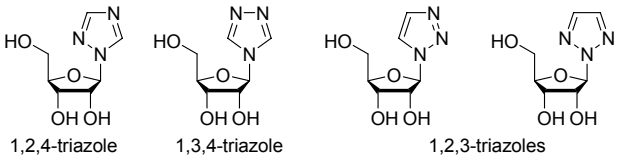


Figure 2.14 Possible triazole nucleosides

From a pharmacological perspective most potent and therefore interesting are the 1,2,4-triazole nucleosides. Ribavirin is such a 1,2,4-triazole nucleoside and has been discovered by Witkowski and Robins in 1972 (Figure 2.15).[54,55] Since the discovery of ribavirin a large

range of analogues has been synthesised. However only a few display a similar biological activity as ribavirin, which is still the most potent one.

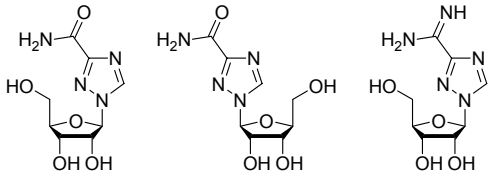
2.2.4.1 1,2,4-triazole nucleosides

Ribavirin is a broad-spectrum antiviral drug active against a number of DNA and RNA viruses. It is one of the few antiviral drugs in clinical use effective against viruses other than HIV and herpes.[56] First, ribavirin was approved in its aerosol form for the treatment of respiratory syncital virus infection in children with respiratory distress.[57,58] Later ribavirin, in combination with interferon-α, was also approved for the treatment of chronic HCV, however the success rate of this therapy is limited.[59,60] Although use of pegylated interferon in combination with ribavirin improved the treatment considerably,[61] clinical use of ribavirin has been overshadowed by its side-effect: haemolytic anemia upon long-term administration.[62]

Ribavirin mimics the configuration of *e.g.* adenosine or guanosine. This makes it possible to target viral and host enzymes that utilise these natural nucleosides as target or co-factor. In addition, indirect effects like inhibition of IMPDH resulting in a decrease of the guanine nucleotide pool,[63] may account for the activity of ribavirin as well. Ribavirin acts as a prodrug, which after entering the cell, is phosphorylated by deoxyadenosine kinase and/or adenosine kinase resulting in the active ribavirin 5'-phosphate.[64] The ribavirin 5'-monophoshate analogue shows a similar *in vitro* broad-spectrum antiviral activity compared to the parent compound. The ribavirin di- and tri-phosphate analogues only showed activity against Type 1 HSV.[65] Ribavirin can be synthesised via reaction of 1,2,4-triazole-3-carboxylate with tetra-O-acetyl-β-ribofuranose at 160°C in the presence of bis(*p*-nitrophenyl)phosphate and subsequent reaction with ammonia in methanol resulting in ribavirin in 70 % yield.[54] In addition enzymatic syntheses are known for ribavirin, where a natural nucleoside with the help of an enzyme is transformed into ribavirin. The natural nucleosides that can be used are orotidine,[66] uridine or cytidine,[67] inosine[68] or guanosine.[69]

The only two 1,2,4-triazole nucleosides that show similar activity as ribavirin are levovirin[70] and viramidine[71] (Figure 2.15). Levovirin is the L-enantiomer of ribavirin (Figure 2.15) and has been synthesised in 2000 by Averett and co-workers.[70] It has similar immunological properties (type 1 cytokine-enhancing activity)[70] as ribavirin, but showed no *in vitro* antiviral activity and no toxicity. This may be explained by the fact that levovirin is not efficiently phosphorylated.[72] The immunomodulatory properties however, seem important as potential HCV drug and therefore Levovirin is now in phase II clinical trials.[73] A disadvantage is that levovirin is only partially absorbed, which results in relatively poor oral bioavailability. Levovirin valinate hydrochloride (R1518), a 5'-valine derivative

addresses this problem. After uptake, R1518 is almost quantitatively hydrolysed to levovirin,[74] which makes it an interesting HCV therapeutic.

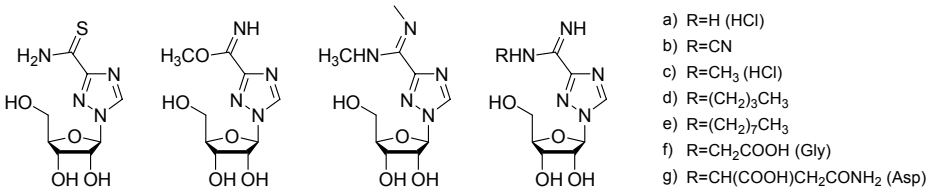


53 **54** **55**

Figure 2.15 Biologically active 1,2,4-triazole nucleosides

Viramidine is the carboxamidine analogue of ribavirin and has been discovered and synthesised in 1973 by Witkowski and Robins.[71] Viramidine acts primarily as prodrug of ribavirin and is converted to ribavirin by adenosine deaminase.[75] Viramidine has some advantages, first it is not taken up by red blood cells like ribavirin, which decreases the potential to cause haemolytic anemia.[76] Second, viramidine is better liver-targeting than ribavirin,[77] while the liver is the main viramidine to ribavirin conversion site. These two advantages make viramidine interesting for the treatment of HCV for which it is currently in phase III of clinical trials.[78] Furthermore, viramidine has been tested for the treatment of human influenza virus infections, while being as active as ribavirin, the toxicity of viramidine is much lower.[79]

Many other analogues have been synthesised, however only a few showed similar bioactivities as ribavirin, viramidine or Levovirin. Sidwell *et al*. synthesised the thio-analogue of ribavirin (**56**), however this compound showed only good activity against Type I HSV.[71] Robins *et al*. published in 1992 the synthesis of some N-carboxamidine substituted analogues of viramidine. Some of these exhibit interesting *in vitro* activities (Figure 2.16).[80] 1,2,4-Triazole **57** showed significant *in vivo* activity against murine leukemia L1210,[81] although its *in vitro* activity was restricted to the bunyaviruses PT and SF and dengue-4-virus. The observed IC_{50} values were comparable to those of ribavirin. 1,2,4-Triazole **58**, dimethylated viramidine, showed similar activity as ribavirin, except that the IC_{50} values of **58** were higher for some viruses, like bunyaviruses PT and SF and VV.

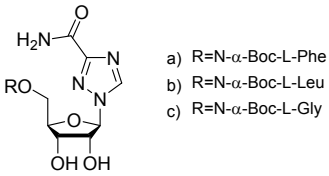


56 **57** **58** **59a-h**

Figure 2.16 Tested viramidine analogues

Other analogues of viramidine, the 1,2,4-triazole nucleosides **59b-h** showed a range of antiviral activities. In general these analogues showed no activity against flaviviruses, an alphavirus or HIV-1. The most potent antiviral nucleoside is **59d** with comparable activity to viramidine against bunyaviruses PT and SF.[80]

Nikolova *et al.* reviewed the antiviral activity of some 5'-amino acid esters of ribavirin (**60**).[82] The activity of these amino acid esters **60** was tested against FPV (influenza virus A), NDV (Newcastle disease virus) and PsRV (pseudorabies virus). Compound **60a** showed activity against FPV and **60b** and **c** against NDV, however these amino acid esters are not as potent as ribavirin. The lower antiviral activity may be related to incomplete hydrolysis under the *in vitro* conditions.

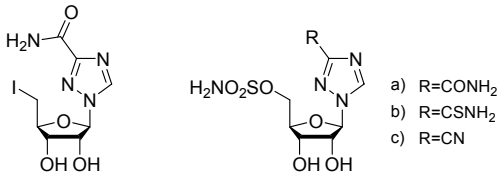


60

Figure 2.17 Amino acid esters of ribavirin

Recently, Cui *et al*. published the synthesis and antiviral activity of 5'-modified 1,2,4-triazole nucleosides with a 5'-azide, amine, hydrogen or iodine functionality.[83] However, only one compound (**61**; Figure 2.18) demonstrated a weak protection against viral infection (IC_{25}=0.4 μmol/mL). No cytotoxicity was observed at this concentration. In addition, Keithly reported antiparasitic activity of some sulfamoyl triazole nucleosides **62a-c** (Figure 2.18).[84] All compounds have been evaluated *in vitro* against five organism, *L. donovani*, *T. cruzi*, *T. gambiense*, *G. lamblia*, *T. vaginalis*. Sulfamoyl triazole nucleoside **62a** proved most active and showed good ED_{50} against *L. donovani* and *T. gambiense*. Because of its good *in vitro* activity against *T. gambiense*, **62a** was tested *in vivo* against *T.*

brucei, however only moderate activity was observed. In addition **62a** showed good activity against visceral leishmaniasis.

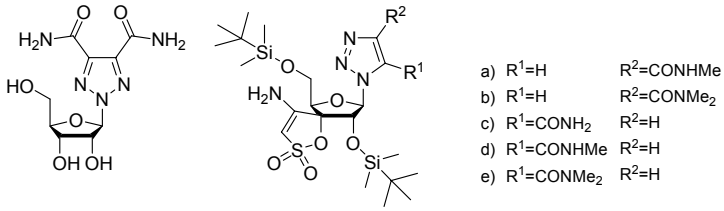


61 **62**

Figure 2.18 5'-Iodo analogue and sulfamoyl nucleosides

2.2.4.2 1,2,3-Triazole nucleosides

1,2,3-Triazole nucleoside **63** and **64** (Figure 2.19) have been prepared and tested for their biological activity.[85,86] The 1,2,3-triazole nucleoside **63** exhibited interesting cell growth inhibition against leukemia K562 and HL-60 cells with comparable activity to ribavirin.[85] Furthermore, the TSAO-triazole nucleosides (**64**) gained interest because of their anti HIV-1 activity.[86] TSAO stands for the modified ribose ring with two TBS groups and one spiro group. 1,2,3-Triazole-TSAO nucleosides **64a-e** showed good anti HIV-1 activity comparable to the TSAO-thymine.[86]

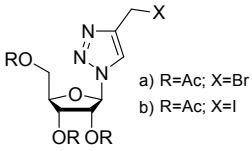


63 **64**

Figure 2.19 1,2,3-Triazole nucleoside

Finally, de las Heras *et al*. reported good cytostatic activity of some N-ribosyl-halomethyl-1,2,3-triazoles **65-67** (Table 2.1).[87] The cytostatic activity increased with $-CH_2X$ alkylating ability, i.e. $-CH_2OH < CH_2Cl < CH_2Br < CH_2I$. The *in vitro* experiments revealed that the type of the sugar ring (furan or pyran), its size (pentose or hexose), or its stereochemistry (ribo or gluco) are not of great importance. The cytostatic activities are similar having the same halomethyl moiety and protecting group. The protecting group is important, *e.g*. the cytostatic activities of acetyl protected sugars are at least 10 times less than benzoyl protected sugars. These triazole nucleosides can be synthesised via the click reaction using the ribosylazide and the appropriate acetylene.

Table 2.1 Cytostatic active 1,2,3-triazole nucleosides



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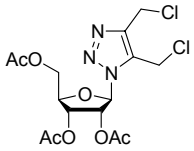

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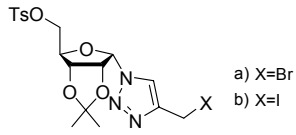


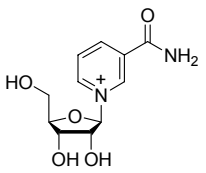
67

Compound	HeLa cells ED_{50} (μg/mL)
65a	5.0
65b	3.5
66	2.5
67a	10.0
67b	10.0

2.3 *Nucleoside analogues with a 6-membered ring nucleobase*

2.3.1 *Pyridine nucleosides*

Only a limited number of biologically active pyridine nucleosides have been described in literature. Perhaps, the most interesting one is nicotinamide riboside (**68**), which is a precursor of nicotinamide mononucleotide. Nicotinamide ribotide is a component for both chemical[88] and enzymatic[89] preparation of nicotinamide adeninedinucleotide (NAD^+, **69**) (Figure 2.20). The importance of NAD is well known; NAD is a co-factor in numerous enzyme-catalysed redox reactions in living organisms and plays a fundamental role in cellular metabolic processes. Therefore, it is of great importance that the level of NAD is regulated and maintained for cellular survival.[90]



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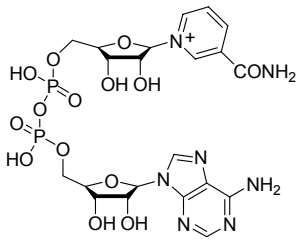
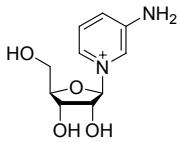


69

Figure 2.20 Nicotinamide and NAD^+

Another important pyridine nucleoside is 3-aminopyridine riboside (**70**). This compound in contrast to other nicotinamide ribose analogues, like 3-acetylpyridine riboside, 3-thionicotinamide and 3-pyridine-aldehyde riboside inhibits *Haemophilus influenza*.[91] Compound **70** is taken up by its NAD-processing and nicotinamide riboside route and in this way inhibits *H. influenza*. Compound **70** was also tested in other species of the family *Pasteurellaceae*, *Pasteurella multocida* and *Actinobacillus actinomycetemcomitans*. However no inhibitory activity was observed, which makes **70** an anti-infective agent with a very narrow host range.[92]

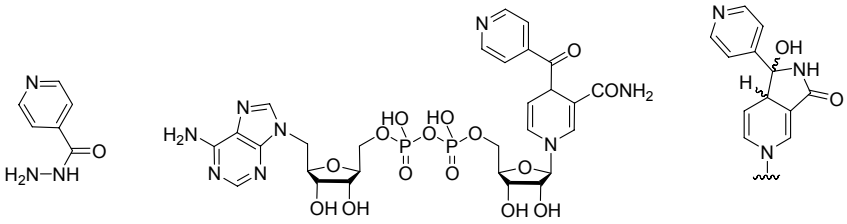


70

Figure 2.21 3-aminopyridine riboside

3-Aminopyridine nucleoside (**70**) is synthesised via a two-step enzymatic degradation starting with the corresponding dinucleotide. The dinucleotide is treated with a snake venom nucleotide pyrophosphatase resulting in the mononucleotide and subsequently converted into the nucleoside with a prostatic acid phosphatase.[93]

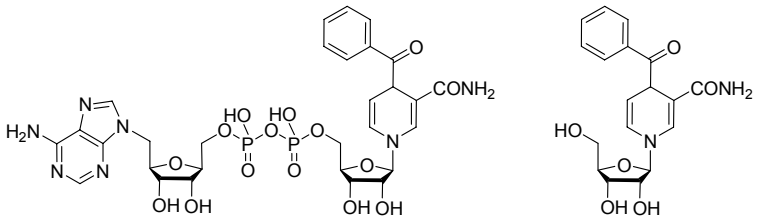
Finally, related to NAD **69** is isoniazid-NAD complex (Figure 2.22). This complex has been proposed as the metabolite responsible for antituberculosis activity of isoniazid (INH; **71** in Figure 2.22).[94] It structure results from binding of the isonicotinoyl radical at C4 of the nicotinamide moiety of NAD (**72**)[95] with further possible debated cyclisation to form a cyclic hemiamidal derivative (**73**).[96] It still unclear whether **72** or **73** is responsible for its anti tuberculosis activity.



71 **72** **73**

Figure 2.22 isoniazid (INH; **71**), INH-NAD complexes **72**-**73**

A benzoylhydrazide (BH) can form the same complex (BH-NAD; **74** in Figure 2.23) and in this way was shown to have the same activity.[97] On this basis, Bernadou *et al*. synthesised benzoyl-1,4-dihydronicotinamide ribonucleoside **75** and reported that this nucleoside does not show any activity against biological targets of isoniazid.[98]

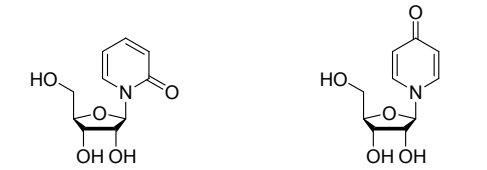


74 **75**

Figure 2.23 BH-NAD and benzoyl-1,4-dihydronicotinamide riboside (**75**)

2.3.2 *Pyridinone nucleosides*

Pyridinone nucleosides can have two different nucleobases, a pyridine-2-one or a pyridine-4-one. Pyridine-4-one nucleosides are scarce and do not show much interesting activities, while pyridine-2-one nucleosides do possess some interesting activities.



Pyridin-2-one nucleoside Pyridin-4-one nucleoside

76 **77**

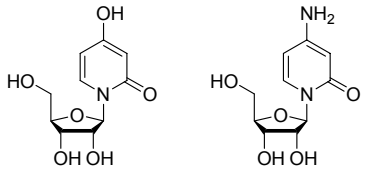
Figure 2.24 Different pyridinone nucleosides

2.3.2.1 *Pyridin-2-one nucleosides*

The most interesting pyridin-2-one nucleoside is 3-deazauridine (3-DU; **78**).[99] This is a broad-spectrum therapeutic, showing *in vitro* activity against several tumour cells, like leukemia L1210 and L1210/AraC (also *in vivo* activity), Ehrlich ascites and Gross leukemia, several RNA viruses, *e.g*. Rhinovirus 1A and 13, coxsachievirus type A21, PR-8 influenza virus, reoviruses type 1, 2 and 3 and SA11 virus, *E. Coli* and *Streptococcus faecium*.[100] Biochemical studies show that 3-DU, after its uptake and intracellular conversion to 3-DU triphosphate, competitively (with uridine triphosphate) inhibits cytidine triphosphate synthetase. This enzyme catalyses the formation of cytidine 5'-triphosphate from uridine-5'-triphosphate.[100c,101] Since 3-DU as a single agent showed only minimal

clinical effectiveness on acute leukemia, there was a lack of interest in clinical investigations using this drug. However, combination therapy drew more attention, resulting in synergistic effects with e.g. cytosine arabinoside[102] or 5-aza-2'-deoxycytidine.[103]

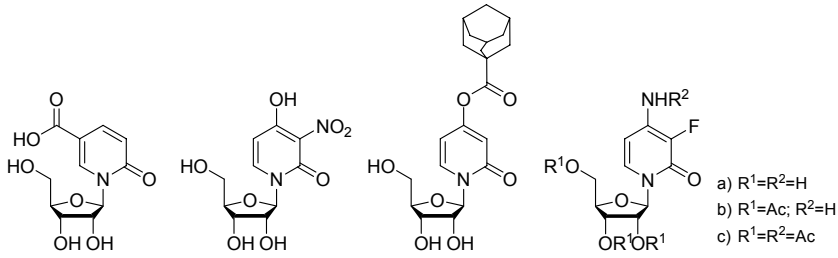
Another interesting pyridine-2-one nucleoside, 3-deazacytidine (3-DC; **79**), was found to be active against L1210 leukemia, *E. coli*, *Streptococcus faecium*, Ehrlich ascites carcinoma, rhino virus 1A, 13 and 56, influenza A & B, parainfluenza virus 1 and vesicular stomatis viruse.[100c,104]



78 **79**

Figure 2.25 3-deazauridine (3-DU; **78**) and 3-deazacytidine (3-DC; **79**)

A few other pyrimidin-2-one nucleosides showed interesting activities. The 5-carboxylic acid pyridin-2-one nucleoside **80** showed significant success in the treatment of adjuvant-induced arthritis in rats.[105] 3-Nitro-3-deazauridine (**81**) showed a much broader spectrum of activity. It is active against a range of RNA virus families, paramyxoviruses, picornaviruses, rhabdoviruses, togaviruses, bugaviruses and rhinovirus type 34.[106] 4-Adamantoyl-3-deazauridine (**82**) showed antitumour activity against leukemia L1210 both *in vitro* and *in vivo*, even better than 3-DU and 3-DC.



80 **81** **82** **83**

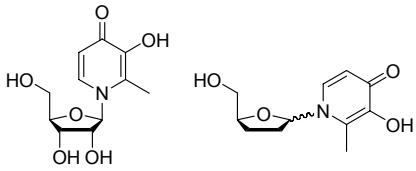
Figure 2.26 Different other pyridin-2-one nucleosides

Finally, three 3-fluoro-3-deazacytidine derivatives (**83a-c**) have been reported by Cook *et al*. in 1987, which all show *in vitro* activity against L1210 leukemia tumour cells in the same range as 3-DC. **83a-b** have been shown to possess also *in vivo* activity.[107] Later *Teepe*

et al. reported the *in vivo* activity of **83a** against P338 leukemia tumour cells and the *in vitro* activity against rhinovirus type 34.[106b]

2.3.2.2 *Pyridin-4-one nucleosides*

Only two biologically active pyridin-4-one nucleosides have been reported (**84** and **85**; Figure 2.27). In 1984 Marquez *et al*. reported the synthesis and the *in vitro* activity of **84** against P338 leukemia tumour cells, while *in vivo* a low activity was observed.[108] Recently, Camplo *et al*. reported the moderate antiviral activity of **85** against HSV-1 and 2.[109]

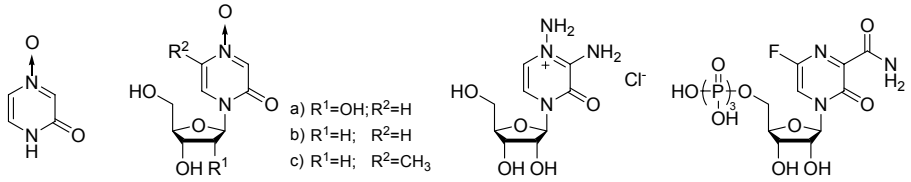


84 **85**

Figure 2.27 Several pyridin-4-one nucleosides

2.3.3 *Pyrazine nucleosides*

Interesting pyrazine nucleosides are limited to only a few examples. Three biologically active pyrazine nucleosides are the ribose analogues (**87a-c**) of emimycin (**86**), which is a natural antibiotic that was isolated from *Streptomyces* No. 2020-I.[110] Emimycin nucleoside **87a** has shown to possess only moderate antibacterial activity against *S. faecium* and *E. Coli*, while being a good anticoccidial agent against several species of chicken *Eimeria*.[111] 2'-Deoxyriboside **87b** shows the reverse activity. While a 100.000 times higher activity against *S. feacium* and *E. Coli* is found, the anticoccidial activity is very low.[111c,112] 5-Methyl emimycin 2'-deoxyriboside **87c** showed low antibacterial activity, while good anti-leukemia activity against L1210 cells was observed.[113]



86 **87** **88** **89**

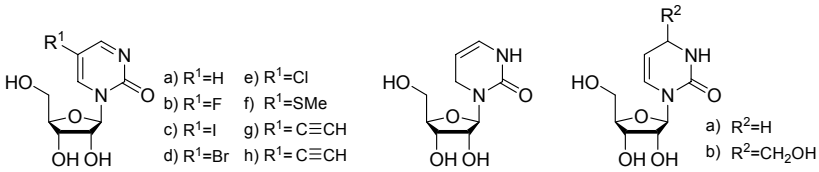
Figure 2.28 Emimycin nucleosides

A further example of pyrazine nucleosides is, *N*-aminopyrazinonium nucleoside **88**, which showed moderate activity as a cytotoxic agent.[114] Finally, triphoshate pyrazine

nucleotide **89** has been found to exhibit potent inhibition of influenza viral polymerase. However, this triphosphate has not been synthesised chemically, but was found to be formed intracellular from the nucleoside by phosphorylation of nucleotidase.[115]

2.3.4 Pyrimidin-2-one nucleosides

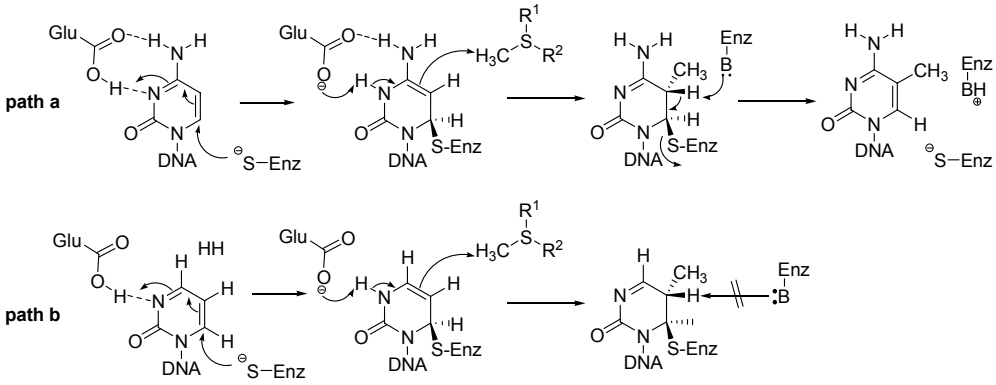
Pyrimidinone nucleosides draw considerable attention as potential anti-cancer drugs. Especially, zebularine (pyrimidin-2-one riboside; **90a** in Figure 2.29), which was originally discovered in 1969 as antibacterial agent, has drawn a lot of attention the last five years as anti-cancer drug.[116] Zebularine (**90a**) targets DNA methyltransferase (DNA Mtase)[117] and cytidine deaminase.[118] DNA Mtases take care of the methylation pattern of DNA and maintain it during every round of DNA replication. Hypermethylation of tumour suppressor genes caused by aberrant activity of DNA Mtase is an important mechanism that contributes to cancer.



90 **91** **92**

Figure 2.29 Pyrimidine nucleosides

The mechanism by which DNA Mtase acts, involves the addition of a protein thiol group (from a cysteine residue) to the C6 position of the target cytidine, which activates the carbon atom at the 5 position allowing reaction with S-adenosyl-L-methionine.[117] This results, after abstraction of the C5-proton, in methylated DNA and free enzyme (Scheme 2.2; path a). The proposed inhibition mechanism of zebularine is depicted in Scheme 2.2 (path b). The absence of the amino group on C4 enhances the nucleophilic attack of the cysteine residue at C6 of the base resulting in a very stable covalent complex. Although a proton is present on C5, the elimination of hydrogen does not take place.[117]



Scheme 2.2 Chemical structure of covalent adduct between DNA Mtase and zebularine

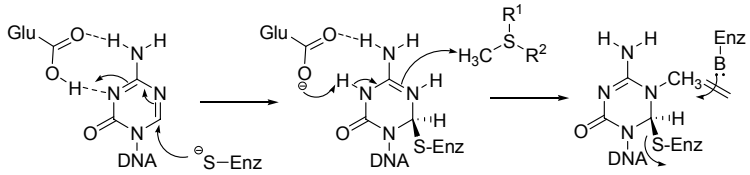
Zebularine preferentially inhibits tumour cells over normal fibroblasts.[119] Furthermore, the response in DNA, RNA, proteins, and cellular growth of tumour cells is higher compared to normal cells. In addition, zebularine is very stable unlike 5-Aza-2'-deoxycytidine (5-Aza-CdR), making oral administration possible. However, a poor bioavailability of zebularine is reported, which can be due to rapid metabolism of absorbed zebularine to uridine and further degradation to uracil. Therefore large doses of zebularine are necessary.[120] A combination therapy with 5-azacytidine (see section 2.3.5.1), which is deactivated by cytidine deaminase and inhibited by zebularine, showed better results than the monotherapy with either of these therapeutics. This makes this approach a serious candidate for clinical use.[121] The mechanism of this drug interaction may involve inhibition of cytidine deaminase by zebularine and inhibition of DNA Mtase by both agents.

Reduction of one of the double bonds of zebularine results in nucleosides **91** and **92a**, which are as potent as zebularine in cytidine deaminase activity. When at C4 a hydromethyl group was introduced (**92b** in Figure 2.29) a 5-fold increase in activity was observed.[118b] Introduction of several other functional groups at C5 resulted in some interesting compounds (Figure 2.29). *E.g*. a SCH_3, ethynyl or propargyl group at C5 resulted in a HSV-1 and/or HSV-2 active compound.[122] The 5-halogen pyrimidinone nucleosides **90b-e** showed all HSV-1 and -2 activity comparable to zebularine or even higher.[118a,123] Barchi *et al*. reported also anti-leukemia activity for 5-fluoro-pyrimidin-2-one nucleoside **90b**.[118d] 5-Iodo-pyrimidin-2-one 2'-deoxyribose (IPdR), the 2'-deoxy analogue of **90c**, is also HSV-1 and -2 active.[124] IPdR is now under preclinical evaluation as prodrug of 5-iodo-2'-deoxyuridine, which is a radiosensitizer.[125]

2.3.5 Azapyrimidine nucleosides

2.3.5.1 5-Azapyrimidine nucleosides

5-Azapyrimidine nucleosides consist mainly of 5-azacytidine (**93a**) and analogues (Figure 2.30), which are all DNA methyltransferase (DNA Mtase) inhibitors. As with zebularine, 5-azacytidine (**93a**) and analogues can form similar covalent complexes with DNA Mtase and in this way inhibit DNA Mtase (Scheme 2.3).[126] The nucleophilic attack of a cysteine residue on C6 is enhanced, because the C6 position is more electrophilic due to the presence of a nitrogen atom at position 5. Due to the absence of a proton on N5 the last step to generate a double bond between positions 5 and 6 cannot take place.

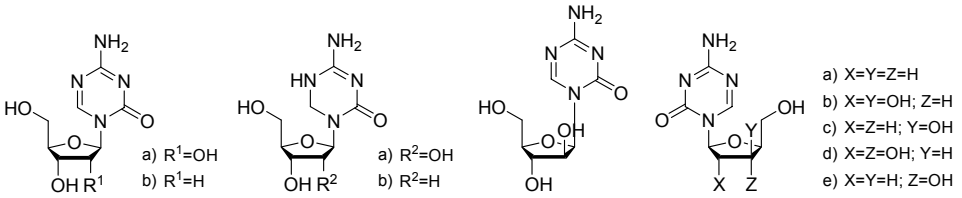


Scheme 2.3 Inhibition of DNA Mtase by 5-azacytidine

5-Azacytidine (**93a**) and 5-aza-2'-deoxycytidine (decitabine; **93b**) were originally tested for their anti-leukemia activity,[127] and were during the 1970s and 1980s used in high, and most of the time toxic, doses to treat leukemia. At that time, 5-azacytidine and decitabine were not recognised as hypomethylating agents yet. It was only in the mid 1990s that 5-azacytidine (**93a**) and decitabine (**93b**) were recognised as DNA MTase inhibitors.[128,129,130] 5-Azacytidine (**93a**) can be incorporated both in DNA and RNA after it is activated to its triphosphate and leads to inhibition of DNA, RNA and protein synthesis.[131] In contrast, decitabine (**93b**), which is a 10-fold more cytotoxic than **93a**, is only incorporated into DNA.[132]

A hydrolytically stable analogue of 5-azacytidine[133] is dihydro-5-azacytidine (**94a**) and shows antitumour activity against L1210 leukemia cells, Friend leukemia, human MX-1 mammary xenograft, murine CD8F mammary tumour, implanted colon 38 tumour and malignant mesothelioma.[134] Dihydro-5-azacytidine (**94a**) targets besides DNA Mtase also RNA synthesis. It is less effective than 5-azacytidines **93a-b** in DNA Mtase inhibition, and a 25-fold higher dose is necessary to obtain the similar therapeutic effects as with **93a**.[135] Phase I and II clinical trials for the treatment of malignant mesothelioma have revealed that **94a** is not effective enough and further clinical research has ceased.[136] The 2'-deoxy analogue **94b** has recently been discovered for the treatment of HIV, however not via

inhibition of DNA Mtase. Compound **94b** uses a different strategy of mutation of the viral genome of HIV resulting in defective viruses.[137]



93 **94** **95** **96**

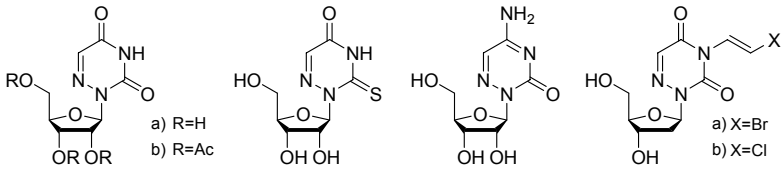
Figure 2.30 5-Azacytidine and analogues

Fazarabine, 1-β-D-arabinofuranosyl-5-azacytosine (**95**), has been synthesised in the late 1970s[138] and has been shown active against L1210 leukemia cell lines,[139] P-338 cell lines,[140] and several human tumor xenografts.[141] Despite, this broad spectrum of activity, clinical trials showed no potent antitumour activity.[142]

L-nucleoside analogs of 5-azacytidine (**96a-e**) have been synthesised and tested for their ability to inhibit HIV, hepatitis B virus (HBV) and Maedi-visna virus (MVV). Only **96a** showed a good anti-HBV activity but no anti-HIV activity,[143] while **96b-e** showed a moderate MVV activity.[144]

2.3.5.2 6-Azapyrimidine nucleosides

This class of nucleoside analogues is dominated by 6-azauridine (**97a**; Figure 2.31). While this compound has been extensively studied, only a few related compounds have shown biological activity. Already in the beginning of the sixties it was shown that 6-azauridine is an anti-psoriasis[145] and anti-neoplastic drug.[146] 2',3',5'-Triacetyl-6-azauridine (**97b**) is the orally active prodrug of 6-azauridine.[147] It can chemically and enzymatically be deacetylated and subsequently metabolised to its 5'-monophosphate nucleotide, which inhibits orotidine 5'-phosphate decarboxylase, an essential enzyme in the biosynthesis of pyrimidines.[148] Although 6-azauridine showed promising cytostatic properties and very low toxicity in animals, only a small effect was observed on human leukemia.[149] In addition a large range of side-effects was observed, *e.g.* fever or joint pain were observed in cases of rheumatoid arthritis.[149a] More recent, 6-azauridine has been evaluated for several viruses, which caused severe outbreaks in the past. 6-Azauridine was proven to be active against yellow fever virus,[150] sandfly fever Sicilian virus,[151] West Nile virus,[152] pathogenic flaviviruses,[153] Chikungunya and Semliki Forest viruses,[154] and HIV-1.[155]



97 **98** **99** **100**

Figure 2.31 Different 6-azapyrimidine nucleosides

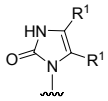
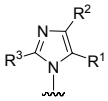
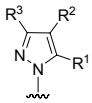
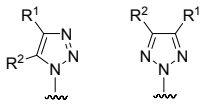
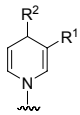
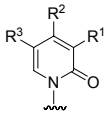
In addition, a few reports on biologically active analogues of 6-azauridine (**97**) have appeared. Olin and co-workers reported the *in vitro* and *in vivo* activity of 2-thio-6-azauridine (**98**) against L1210 leukemia cells.[156] Furthermore, **98** shows also *in vitro* and/or *in vivo* antiviral activity against five RNA viruses,[157] and *in vitro* activity against the West Nile virus.[152] 6-Azacytidine (**99**) was shown to be an antiviral agent too, with a broad spectrum of activity, for example several adeno-, herpes- and influenza- viruses are inhibited.[158] Finally, Scopes *et al*. reported two 6-aza analogues of (*E*)-5-(2-Bromovinyl)-2'-deoxyuridine (BVDU). BVDU is one of the most potent *in vitro* herpes simplex virus type 1 (HSV-1) inhibitors. Although a 500-fold lower activity is observed for **100a-b** compared to BVDU, it is still of significance.[159]

2.4 Conclusion

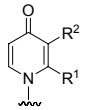
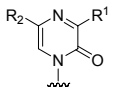
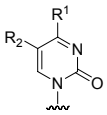
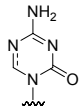
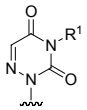
The nucleoside analogues discussed in this chapter show a broad spectrum of activity, different classes show different activities. Table 2.2 summarises the potential therapeutic areas of these nucleoside analogues. The discovery of a new clinical therapeutic application boosts often synthesis of analogues. In this way even more interesting nucleosides have been found; *e.g*. ribavirin used for the treatment of hepatitis C was overtaken by viramidine, because the side-effects were reduced and viramidine was a better liver targeting antibiotic.

In this context, our goal to synthesise dihydropyrimidine nucleosides, as discussed in chapter 1, is relevant for the development of new therapeutics. The related pyrimidine nucleosides (see section 2.3.4) show a broad range of therapeutic action: antitumour, antiviral and antibacterial activity. In order to get dihydropyrimidine nucleosides, first dihydropyrimidines and the 3'-deoxyriboses need to be synthesised.

Table 2.2 Overview of nucleoside analogues and their therapeutic activity

Nucleoside type	Structure base	Therapeutic activity
Imidazole		- immunosuppressant - antiviral - AMPK activator - antitumour - antimetabolic - cytostatic - antifungal - IMP dehydrogenase inhibitor
Imidazolin-2-one		- chitin synthetase inhibitor - antifungal - anti-yeast - HIV reverse transcriptase inhibitor - anti-insecticidal - anti-acaricidal
Pyrazole		- cytostatic activity - antiproliferative - antitumour - anti-influenza
1,2,4-Triazole		- antiviral - immunomodulatory properties - antiparasitic - anti visceral leishmaniasis
1,2,3-Triazole		- antiviral - cytostatic activity
Pyridine		- cell regulation - anti-influenza - anti-infective - anti-tuberculosis
Pyridin-2-one		- antitumour - antiviral - antiarthritis

Continuation of Table 2.2

Nucleoside type	Structure base	Therapeutic activity
Pyridin-4-one		- antitumour - antiviral
Pyrazine		- antibacterial - anticoccidial - antitumour - anti-influenza
Pyrimidin-2-one		- antitumour - antibacterial - antiviral - radiosensitizer
5-Azapyrimidine		- antitumour - antiviral - RNA synthesis
6-Azapyrimidine		- antipsoriasis - antineoplastic - cytostatic activity - antiviral - antitumour

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3

Multicomponent Synthesis of Dihydropyrimidines

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A broad range of differently substituted dihydropyrimidines can be efficiently prepared using a four-component reaction between phosphonates, nitriles, aldehydes and isocyanates. The scope and limitations of this multicomponent reaction are fully described. Variation of all four components was investigated. The nitrile and aldehyde inputs can be varied extensively but variation of the phosphonate input remains limited. An interesting rearrangement leading to phosphoramidates has been observed. Furthermore, the multicomponent reaction seems to be restricted to the use of isocyanates with strongly electron-withdrawing substituents, but an interesting additional exchange reaction under microwave conditions leads to dihydropyrimidines with less electron-withdrawing substituents at *N*3. In addition, a diastereoselective formation of dihydropyrimidines has been observed when using a chiral aldehyde as the input.

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3.1 Introduction

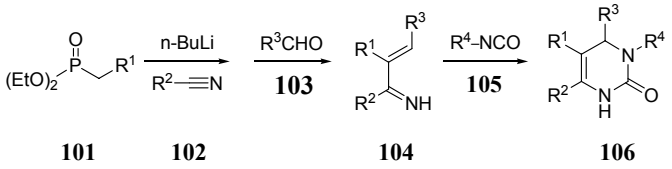
Structurally diverse libraries of small molecules are conveniently generated by combinatorial assembly of different types of building blocks onto a common scaffold. Most approaches rely on a stepwise protocol, but for more complicated skeletons such sequential re-iteration of individual reactions becomes a limiting factor. A more convergent approach is desired that ideally provides the suitably decorated scaffold in a single synthetic operation. Procedures that yield complex molecules by performing multiple reaction steps in which several bonds are formed without isolation of intermediates are commonly referred to as tandem reactions.[1] An important subclass of tandem reactions are the multi-component reactions (MCRs), which are defined as one-pot processes in which at least three easily accessible components are combined to form a single product.[2] In this regard, MCRs are receiving ever-increasing attention as useful tools for parallel syntheses of arrays of structurally diverse products with considerable complexity.[3,4]

Many small synthetic organic molecules with high medicinal potential contain heterocyclic rings. Also, the most potent ligand systems in (transition) metal-mediated (asymmetric) catalysis are often based on heterocyclic cores. The range of easily accessible and suitably functionalised heterocyclic building blocks is, however, surprisingly limited and the construction of even a small array of, for example, 500 relevant heterocyclic compounds is far from trivial. Heterocyclic chemistry therefore continues to attract the attention of medicinal and synthetic chemists,[5] and the development of novel methodologies allowing efficient access to heterocycles efficiently is still highly appreciated. Traditionally, methods based on MCRs have proven quite efficient for the construction of many different types of heterocycles.[4b] For example, the Hantzsch four-component pyridine synthesis, the Bucherer–Bergs three-component thiazole synthesis, and the Biginelli three-component reaction for the preparation of dihydropyrimidines (DHPMs) are all well-known MCRs for the construction of heterocyclic species.[4b] Recently, we have also contributed with several examples in this area.[6,7]

DHPMs are versatile heterocyclic 6-membered ring scaffolds with remarkable pharmacological activities.[8] They are widely used in medicinal chemistry. For example, antiviral, antitumour, antibacterial and anti-inflammatory activities have been reported and DHPMs are also used as calcium channel modulators, α1a-adrenoceptor-selective antagonists and anti-hypertensive agents.[9] The methods hitherto used for the preparation of biologically active DHPMs all involve multi step processes.[10-13] An interesting approach was reported by Elliott and co-workers during their work on the synthesis of Batzelladine A,[14] for which they developed an aza-DA reaction between a dihydro-oxazole and

isocyanates resulting in oxazolopyrimidines.[15] Other well-known approaches to DHPMs use the classical Biginelli 3CR.[8a,16] In the past decade, a series of procedures has been developed to overcome the relatively harsh conditions (EtOH, HCl, Δ) of the original reaction.[17] A serious drawback of these protocols is that only the pharmacologically less important *N*1-substituted and *N*3-unsubstituted DHPM derivatives can be obtained. Additional synthetic manipulations are needed to obtain the biologically active *N*3-substituted DHPMs.[8,18]

The four-component reaction (4CR) depicted in Scheme 3.1 offers an alternative, which largely overcomes these problems and produces the desired *N*3-substituted DHPMs **106** in a single step. In this chapter the tolerance of this reaction to a broad range of nitrile **102** and aldehyde **103** inputs are presented. In addition, an extensive scope study is reported that addresses the influence of the phosphonate (**101**) and the isocyanate (**105**) components on the four component assembly of DHPMs **106**. Steric and electronic factors that direct this MCR are discussed in detail in order to fully define its exploratory power E_N. Also, the use of optically active input has been explored in order to obtain the desired DHPMs **106** diastereoselectively. Finally, ketones are also tested in this new MCR.



Scheme 3.1 Multi component approach to DHPMs.

101a R^1 = H
101b R^1 = Me
101c R^1 = Ph
101d R^1 = CH=CH_2
101e R^1 = $PO(OEt)_2$
101f R^1 = CO_2Me

102a R^2 = Ph
102b R^2 = *i*Pr
102c R^2 = 2-Furyl
102d R^2 = CHMe(Et)
102e R^2 = *t*Bu
102f R^2 = *n*Pr

103a R^3 = 4-MeOPh
103b R^3 = 4-ClPh
103c R^3 = Ph
103d R^3 =4-NMe_2Ph
103e R^3 = 3-NO_2Ph
103f R^3 = 4-NO_2Ph
103g R^3 = *i*Pr
103h R^3 = $PhOCH_2OCH_2$
103i R^3 = Me
103j Myrtenal

105a R^4 = Ts
105b R^4 = 4-NO_2Ph
105c R^4 = Ph
105d R^4 = C(O)Ph
105e R^4 = CO_2Me
105f R^4 = CH(Me)Ph

3.2 Results and Discussion

The reaction for the synthesis of DHPMs of type **106** as depicted in Scheme 3.1 is in fact a combination of a Horner-Wadsworth-Emmons reaction and an aza-Diels-Alder reaction (HWE/aza-DA 4CR) and most likely proceeds via 1-azadiene intermediates **104**. These species are conveniently generated by mixing a phosphonate (**101**) with a base (e.g. *n*BuLi) at –78 °C, followed by nucleophilic addition to a nitrile (**102**). The intermediate ketimine is more nucleophilic at carbon than at nitrogen, resulting in a Horner-Wadsworth-Emmons reaction with a third component, an aldehyde (**103**),[7,19] to give 1-azadiene **104**. The mixture is then allowed to warm to room temperature, whereupon addition of an isocyanate (**105**) induces a formal Diels-Alder cycloaddition to give **106** (Scheme 3.1).

3.2.1 Scope study of nitrile, aldehyde and isocyanate inputs

Reaction of diethyl methylphosphonate (**101a**) and tosylisocyanate (**105a**) with aromatic nitriles **102** and aromatic aldehydes **103** proceeds smoothly and efficiently affords the corresponding DHPMs **106a**–**106f** (entries 1–6; Table 3.1) efficiently.

Table 3.1 HWE/aza-DA 4CR of phosphonate **101a** and isocyanate **105a** with nitriles **102** and aromatic aldehydes **103**.

Entry	Nitrile	R^2	Aldehyde	R^3	Yield (%)	DHPM
1	**102a**	Ph	**103a**	4-MeOPh	65	**106a**
2	**102a**	Ph	**103b**	4-ClPh	80	**106b**
3	**102a**	Ph	**103c**	Ph	71	**106c**
4	**102a**	Ph	**103d**	4-Me_2NPh	54	**106d**
5	**102a**	Ph	**103e**	3-NO_2Ph	38	**106e**
6	**102c**	2-furyl	**103a**	4-MeOPh	61	**106f**
7	**102b**	*i*Pr	**103a**	4-MeOPh	73	**106g**
8	**102b**	*i*Pr	**103b**	4-ClPh	65	**106h**
9	**102b**	*i*Pr	**103c**	Ph	55	**106i**
10	**102b**	*i*Pr	**103d**	4-Me_2NPh	49	**106j**
11	**102b**	*i*Pr	**103e**	3-NO_2Ph	60	**106k**
12	**102b**	*i*Pr	**103f**	4-NO_2Ph	55	**106l**
13	**102d**	CH(Me)Et	**103a**	4-MeOPh	49	**106m**
14	**102e**	*t*Bu	**103a**	4-MeOPh	15	**106n**
15	**102f**	*n*Pr	**103b**	4-ClPh	< 5	**106o**

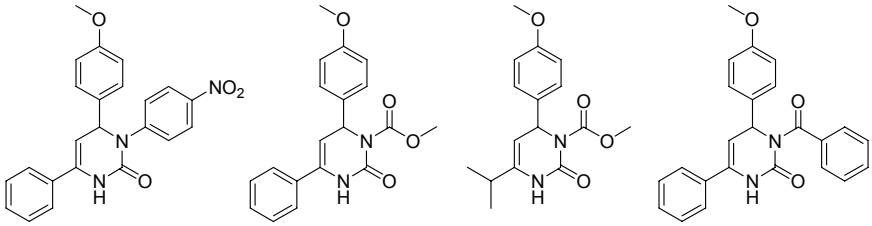
Aliphatic nitriles **102** in combination with aromatic aldehydes **103**, **101a** and **105a** gave modest to good yields of the corresponding DHPMs **106g–106o** (entries 7–15; Table 3.1). When secondary nitriles **102b** and **102d** were used (entries 7–13; Table 3.1) the yields of the corresponding DHPMs **106g–106m** were still satisfying. However, when the sterically more demanding pivalonitrile (**102e**) was used as input (entry 14; Table 3.1) the yield of **106n** was rather poor. Also, use of the primary nitrile **102f** resulted in only traces of the desired DHPM **106o** (entry 15; Table 3.1). In a related study toward the synthesis of α,β-unsaturated ketones via *in situ* generated 1-azadienes, it was reported that primary nitriles are quite prone to dimerisation and polymerisation,[19,20] which may account for the diminished yield of DHPM **106o**.

Besides aromatic aldehydes, aliphatic aldehydes were also used as inputs in the MCR. When these aliphatic aldehydes (**103g**, **103h**, and **103i**) were allowed to react with nitriles **102a–c, 101a** and **105a** only moderate yields of the corresponding DHPMs **106p–106t** could be isolated (Table 3.2).

Table 3.2. HWE/aza-DA 4CR of phosphonate **101a** and isocyanate **105a** with nitriles **102** and aliphatic aldehydes **103**.

Entry	Nitrile	R^2	Aldehyde	R^3	Yield (%)	DHPM
1	**102a**	Ph	**103g**	*i*Pr	35	**106p**
2	**102a**	Ph	**103i**	Me	15	**106q**
3	**102c**	2-furyl	**103g**	*i*Pr	36	**106r**
4	**102b**	*i*Pr	**103g**	*i*Pr	40	**106s**
5	**102b**	*i*Pr	**103h**	$PhOCH_2OCH_2$	21	**106t**

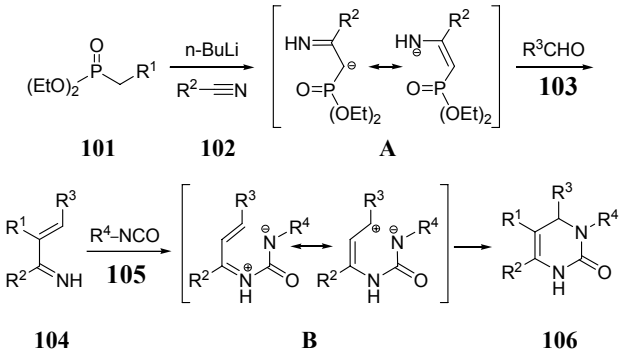
In order to further explore the scope of the HWE/aza-DA 4CR for the synthesis of DHPMs **106** the isocyanate input **105** was also varied (Figure 3.1). Isocyanates are versatile starting materials, which are used in other MCRs as well.[21] Thus, next to **105a**, also isocyanates **105b**, **105d** and **105e** proved successful when reacted with **101a**, **102a**, and **103a** in the presence of *n*BuLi and afforded DHPM **106u–106x** in modest to good yield. Several pharmacological studies show that an ester or amide function at *N*3 or *C*5 in DHPMs like **106** are required for biological activity.[8c] To our satisfaction isocyanate **105e** afforded in this way, pharmaceutically more relevant DHPMs **106v** and **106w** in 70% and 29% isolated yield respectively.



106u (26%) **106v** (70%) **106w** (29%) **106x** (70%)

Figure 3.1 DHPMs **106** formed with various isocyanates **105**.

The synthesis of **106** most likely starts with abstraction of the α-*H* of **101** by *n*BuLi, followed by nucleophilic addition to the nitrile **102** (Scheme 3.2). The intermediate ketimine **A** is more nucleophilic at carbon than at nitrogen, resulting in a Horner-Wadsworth-Emmons reaction with the third component, the aldehyde **103**, to give 1-azadiene **104**. Efficient generation of the resonance stabilised **A** is crucial and strongly depends on the type of nitrile used. The use of nitriles with poorly accessible α-hydrogens as well as aromatic nitriles generally produce the ketimines efficiently,[11b] which is reflected in the yields of DHPMs **106**.

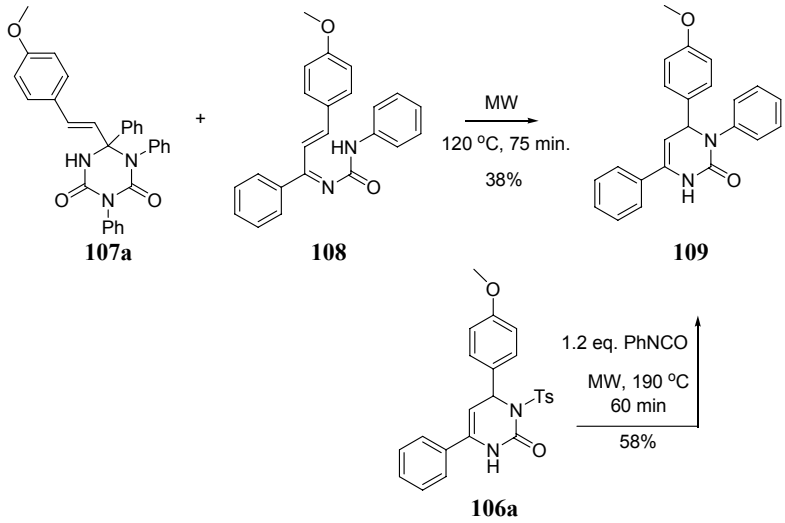


101 **102** **A** **104** **B** **106**

Scheme 3.2 Horner-Wadsworth Emmons aza Diels-Alder four component reaction

Although, a nitrogen at the 1-position of **104** creates a π-deficient diene, which usually shows much lower reactivity towards dienophiles, 1-azadiene **104** must undergo a formal DA reaction with the electron-deficient N=C π-bond of isocyanate **105** in order to afford the observed DHPMs **106**. Normal aza-DA reactions, where an electron-rich (aza)diene reacts with an electron deficient (aza)dienophile, are well-known using 2-azadienes.[22] Similar DA-reactions with 1-azadienes, however often proceed sluggish and are of limited synthetic significance.[23] Usually, the thermodynamic driving force for an (concerted) aza DA reaction of 1-azadienes is, compared to butadienes and 2-azadienes about 200 kcal/mol lower resulting in a much lower reactivity toward dienophiles.[23,24] On the other hand, cycloaddition reactions

involving isocyanates are reported to proceed via a polar stepwise mechanism in almost all cases.[25] In our study a stepwise mechanism for the cyclocondensation to generate **106** is supported by isolation of triazinane dione **107a** and non-cyclised **108**, where **101a**, **102a**, and **103a** were used in combination with isocyanate **105c** (Scheme 3.3). The isolation of the non-cyclised linear precursor **108** strongly supports a stepwise cycloaddition of the corresponding 1-azadiene intermediate with **105c**.[26] These observations suggest that the final cyclocondensation proceeds through stabilised intermediates **B** although a concerted DA cyclisation for the MCRs using more electron-deficient TsNCO cannot be excluded.[27] Thus, non-cyclised **108** can be formed from intermediate **B** via a 1,3-H-shift. Formation of triazinane dione **107a** can be rationalised by addition of a second equivalent of PhNCO to **B** followed by ring closure to the thermodynamically favoured six-membered heterocycle.



107a **108** **109** **106a**

Scheme 3.3 Synthesis of DHPMs **109** with less electron withdrawing R^4-substituents

The HWE/aza-DA approach to form DHPMs **106** directly, seems confined to the use of isocyanates with relatively strongly electron-withdrawing substituents R^4. Strong electron withdrawing groups R^4 on the isocyanate favour aza-DA of the initially formed 1-azadiene **104** to form the corresponding DHPM **106**. However, the application of **105** with less electron-withdrawing R^4 can successfully lead to DHPMs using microwave (MW) conditions. To our satisfaction, **108** could be cyclised under MW-conditions at 120 °C in THF as the solvent and the desired DHPM **109** could be isolated in 38% yield (Scheme 3.3). Moreover, we also observed an interesting exchange reaction under MW-irradiation. When DHPM **106a** (R^4=Ts, y = 65%) is heated together with isocyanate **105c** (R^4=Ph) at 190 °C in dioxane for 1h in a monomode MW oven, the DHPM **109** was formed in 58% yield (Scheme 3.3).

3.2.2 Scope study of phosphonate input

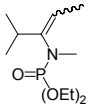
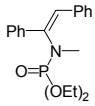
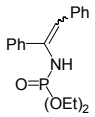
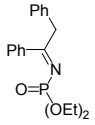
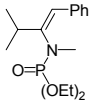
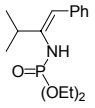
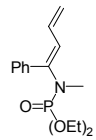
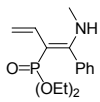
Another point of diversity in the DHPM-scaffold (**106**) is R^1, which is introduced via the phosphonate input **101**. Therefore, we decided to investigate the application of different phosphonates in the HWE/aza-DA 4CR. Phosphonates **101** were prepared by way of the Michaelis–Arbuzov reaction.[28] Thus, reaction of triethyl phosphite with appropriate halides under controlled microwave heating in a monomode reactor afforded phosphonates **101a–d** in very good yields.[29]

Table 3.3. HWE/aza-DA 4CR of phosphonates **101a** and **101b**, nitriles **102a-c**, aldehyde **103** and isocyanate **105a**.

Entry	Phosphonate	R^1	Nitrile	R^2	Yield (%)	DHPM
1	**101a**	H	**102a**	Ph	65	**106a**
2	**101a**	H	**102c**	2-furyl	61	**106f**
3	**101a**	H	**102b**	*i*Pr	73	**106h**
4	**101b**	Me	**102a**	Ph	35	**106y**
5	**101b**	Me	**102c**	2-furyl	90	**106z**
6	**101b**	Me	**102b**	*i*Pr	--	--

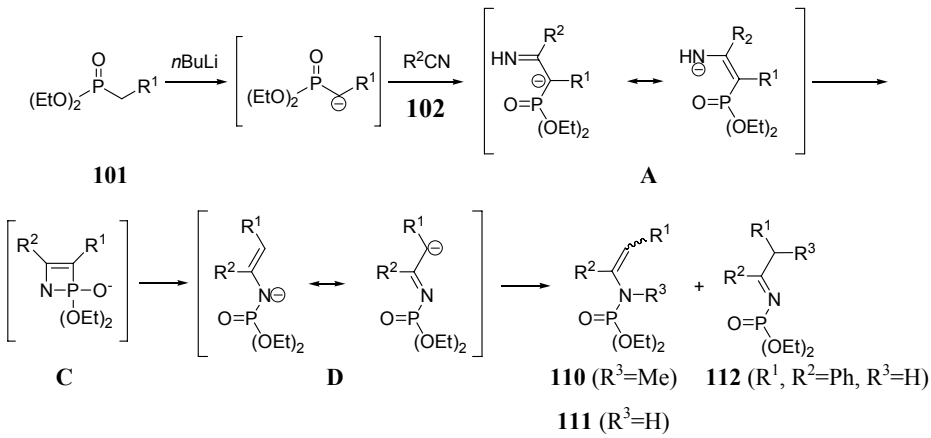
Phosphonates **101a–d** were used in combination with a small set of nitriles (**102a–c**), anisaldehyde (**103a**) and tosylisocyanate (**105a**). The results are summarised in Table 3.3 and Table 3.4. The use of phosphonate **101b** instead of **101a** in combination with aromatic nitriles **102a/102c**, **103a** and **105a** results in moderate to good yields of the corresponding DHPMs **106y** and **106z** (entries 4 and 5, Table 3.3), although the yield of **106y** is considerably lower than that of **106z**. Moreover, when the aliphatic nitrile **102b** is reacted with **101b, 103a** and **105a** no DHPM could be detected (entry 6; Table 3.3). The other two synthesised phosphonates **101c** and **101d** also failed to give DHPMs in the HWE/aza-DA 4CR. To clarify the course of the reaction, the consumption of phosphonates **101b**, **101c** and **101d** by either **102a** or **102b** was studied in more detail using ^{31}P NMR spectroscopy. For the reaction involving **101b**, this revealed that although initial deprotonation with *n*BuLi proceeds smoothly, the resulting anion reacts rather slowly with **102b**. Even after stirring the reaction mixture at room temp. overnight, considerable amounts of unreacted **101b** were still detected. Nevertheless, formation of the corresponding intermediate of type **A** (Scheme 3.2) most likely took place. When the reaction mixture was quenched with MeI (no products could be identified with H_2O) the intermediate was indeed trapped. A mixture of products was formed; the main products were identified as the phosphoramidates (*E*)- and (*Z*)-**110a** (37%, entry 1; Table 3.4).

Table 3.4. Reaction of phosphonates **101b-d** with nitriles **102a** and **b**.[a]

Entry	Phosphonate	Nitrile	Quench method	Products		Recovery of **101**
1	**101b**	**102b**	MeI		**110a** (37%) *E:Z*=1:6	56%[b]
2	**101c**	**102a**	MeI		(*Z*)-**110b** (100%)	--
3	**101c**	**102a**	H_2O		**111a** (58%) *E:Z*=1:1.5	--
					**112** (42%)	
4	**101c**	**102b**	MeI		(*Z*)-**110c** (20%)	7%[c]
5	**101c**	**102b**	H_2O		(*Z*)-**111b** (25%)[d]	7%[c]
6	**101d**	**102a**	MeI		(*E*)-**110d** (37%)	--
					(*E*)- **113** (63%)	

a) After reaction with nitrile was complete (entries 2, 3 and 6) or after stirring at room temp. overnight (entries 1, 4 and 5), the reactions were quenched by either MeI or H_2O and the product composition was analysed with ^{1}H and ^{31}P NMR, see experimental for more details; b) A third phosphoramidate (6%) was identified as well, see reference 30; c) Another phosphonate (10-20%) was identified next to residual **101c**, see reference 31; d) Phosphoramidate (*Z*)-**111b** decomposes quickly.

Also, when phosphonate **101c** was used in combination with **102a, 103a** and **105a** in the 4CR, no corresponding DHPM was formed at all. However, if after initial deprotonation and addition of **102a** the reaction was quenched using MeI, the sole identifiable product was phosphoramidate **110b** (quant., entry 2; Table 3.4). On the other hand, when the same reaction mixture was quenched with water instead of MeI (entry 3; Table 3.4) the phosphoramidates (*E*)- and (*Z*)-**111a** (58%) together with the imine **112** (42%) were found, as concluded on the basis of the NMR data. Next, the reaction of **101c** with isobutyronitrile (**102b**) was investigated (entries 4 and 5; Table 3.4). Although initial deprotonation seemed to proceed smoothly, the resulting anion reacted only relatively slowly with **102b**. After stirring at room temperature overnight, 7% of unreacted **101c** remained. Additionally, some 20-25% of the corresponding phosphoramidates (*Z*)-**110c** and (*Z*)-**111b** were identified when MeI and water, respectively, were used to quench the reaction. Finally, when diethylallyl phosphonate **101d** was deprotonated in a similar fashion as above with *n*BuLi, the resulting anion was found to react with benzonitrile **102a**. After quenching with MeI two main products, (*E*)-**110d** and **113** were identified in a 1:2 ratio, respectively (entry 6; Table 3.4). The process depicted in Scheme 3.4 can rationalise the formation of phosphoramidates **110** and **111** as well as the formation of imine **112**. After initial deprotonation of the phosphonates **101b–d**, the resulting anions react with nitriles **102a** or **102b** to form the corresponding stabilised intermediates **A**. Then, **A** rearranges to **D**, which is also stabilised by resonance. Ultimately, the observed rearranged products are formed by reaction of **D** with MeI (**110**) or water (**111** and **112**). The rearrangement of **A** → **D** may proceed via **C**, which is a four-membered N,P-containing ring analogous to the oxaphosphetane-intermediates that play a role in the usual HWE reactions.[32]



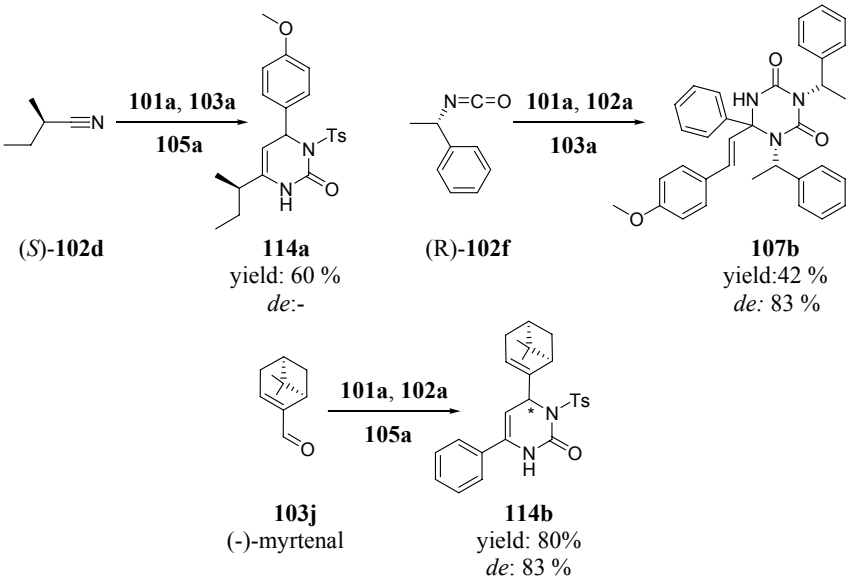
Scheme 3.4 Mechanism for formation of phosphoramidates **110**–**112**.

Efficient formation of **A** depends on the stability of the initial anion in combination with the accessibility of the α-proton of the nitrile input. Furthermore, the intermediate **A** derived from **101b** is less stable than the corresponding anion derived from **101c** or **101d**.[33] Consequently, DHPMs **106** can still be formed upon reaction of the anion of **101b** with aromatic nitriles **102** followed by a quick HWE/aza-DA process with **103a** and **105a** (entries 4 and 5; Table 3.3). On the other hand, after deprotonation of the phosphonates **101c** and **101d** the anion reacts smoothly with the nitrile input to form **A** but then fast rearrangement to **D** is preferred, which ultimately leads to products **110**, **111** and **112**. Only one precedent for a similar rearrangement exists in literature.[34] Two commercially available phosphonates **101e** and **101f** were treated with *n*BuLi and a nitrile in a similar manner as described above. Deprotonation was successful in both cases, but resulted in a very stable anion that did not react with benzonitrile **102a** at all and no rearranged products were observed. Also, one-pot reaction of either **101e** or **101f** with **102a**, **103a** and **105a** did not give the corresponding DHPM as a product. Addition of crown ethers or the use of HMPA as co-solvent did not result in reaction of the anion. Also, the use of alternative bases, like KO*t*Bu or LDA did not result in any reaction of the anion **A** of **101e** or **101f** with **102a**.

3.2.3 Chiral starting materials as input in MCR

The biological activity of DHPMs depends on the absolute configuration at the C4 centre, whereby the orientation of the substituent acts as a molecular switch between agonist and antagonist activities.[9c] A stereoselective synthesis of optically pure DHPMs is therefore of great relevance for medicinal applications of these compounds. Although not comparable to the HWE/aza-DA 4CR, stereoselective formation of DHPMs via the classical Biginelli-3CR has been reported. Dondoni and co-workers achieved good *de* values by using *C*-glycosylated inputs,[35] and very recently an enantioselective Biginelli 3CR was reported using a chiral Ytterbium catalyst.[36] In order to induce chirality in our HWE/aza-DA 4CR, three reactions were performed with different optically pure inputs (Scheme 3.5). First, a reaction was performed with the commercially available optically pure nitrile (*S*)-(+)-**102d** in combination with **101a**, **103a** and **105a**. This gave a 1:1 diastereomeric mixture of **114a** in 60% yield. When optically pure isocyanate (*R*)-**105f** was combined with **101a**, **102a**, and **103a**, the corresponding DHPM was not formed. Instead triazinane dione **107b** was isolated in 42 % yield and a 1:1.5 mixture of diastereomers. Formation of triazinane diones has been observed previously for less reactive isocyanate inputs (Scheme 3.3) This interesting reaction will be further explored and will be subject of a forthcoming paper. However, when the commercial (-)-myrtenal **103j** was used as the aldehyde input (with **101a**, **102a** and **105a**) the corresponding DHPM **114b** was formed in 80% yield as a 11:1

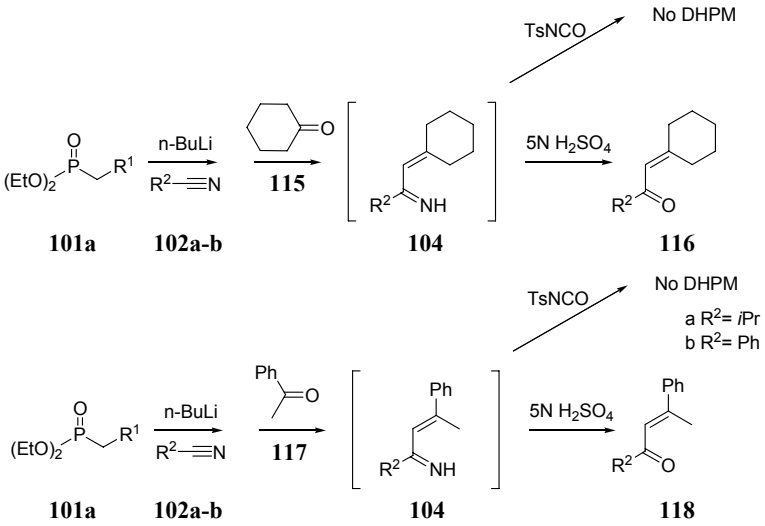
diastereomeric mixture, which is comparable to what Dondoni *et al*.[35] found when they used two chiral inputs in the Biginelli 3CR.



Scheme 3.5 Use of chiral inputs in the HWE/aza-DA 4CR.

3.2.4 Application of ketones in HWE/aza-DA 4CR

In order to further explore the scope of the four-component HWE/aza-DA reaction some ketones were also used as inputs instead of aldehydes. When cyclohexanone (**115**) or benzophenone (**117**) was combined with **101a**, **105a** and either **102a** or **102b**, the corresponding DHPM was not formed (Scheme 3.6). However, when the reactions were quenched with sulphuric acid prior to addition of **105a**, the α,β-unsaturated ketones **116** and **118** were detected.[37] This indicates that the HWE-process took place and that the corresponding 1-azadiene intermediates did form. Apparently, these are not reactive enough to undergo subsequent cycloaddition upon *in situ* treatment with **105a** to give the desired DHPM analogues.



Scheme 3.6 Use of ketones in the HWE/aza-DA 4CR.

3.3 Conclusions

The four component HWE/aza-DA approach to synthesise a variety of DHPMs has been extensively explored. A broad range of different R^2 (nitrile) and R^3 (aldehyde) substituents can be introduced on the DHPM scaffold via this procedure. However, variation of R^1 (phosphonate) remains limited. Next to R^1=H, R^1=Me is tolerated depending on the type of nitrile applied, but with other R^1 groups in the phosphonate input a rearrangement reaction is observed that results in phosphoramidates as the main product. The R^4 substituent can be varied, although direct formation of DHPMs is restricted to the use of isocyanates that bear a strongly electron-withdrawing R^4 substituent. However, less electron-withdrawing R^4 substituents can be easily introduced by heating the initially formed DHPM with appropriate isocyanates under microwave conditions. Furthermore, we have demonstrated that, in particular, the use of optically pure aldehydes as input results in diastereoselective formation of the corresponding DHPM.

3.4 Acknowledgements

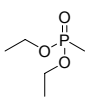
We want to thank Dr. Marek Smoluch and Maarten Posthumus from Wageningen University for measuring the HRMS samples.

3.5 Experimental section

3.5.1 General

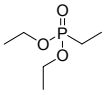
All reactions were carried out under dry nitrogen or argon. ^{1}H, ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 (250.13, 62.90 and 101.25 MHz, respectively) or a Bruker MSL 400 (400.13, 100.61 and 161.98 MHz respectively) spectrometer; chemical shifts (δ) are given in ppm, internally referenced to residual solvent resonances (^{1}H: δ 7.29 ppm, ^{13}C: δ 77.0 ppm). Column chromatography was performed on Baker 7024-02 silica gel (40 μ, 60 Å) with petroleum ether (PE, boiling range 40-60 °C) and ethyl acetate (EA) as eluents. Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC plates were visualised under UV light or by treatment with an anisaldehyde solution (6 ml p-anisaldehyde, 7 ml acidic acid and 7 ml sulphuric acid in 120 ml ethanol). High-resolution mass spectra (HRMS, EI) were recorded on a Finnigan Mat 900 spectrometer at 70 eV. IR spectra were recorded on a Mattson 6030 Galaxy spectrophotometer and are reported in cm^{-1}. Melting points were measured on a Stuart Scientific SMP3 melting point apparatus and are uncorrected. Diastereomeric excess (*de*) was determined on a Shimadzu Prominence, equipped with a DAD detector and a Shimadzu SIL-20A auto-injector. Optical rotation was determined with an AA-10 automatic polarimeter from Optical Activity Ltd. Microwave reactions were performed in a monomode microwave (MW) reactor equipped with an autosampler (CEM explorer). The temperature was controlled throughout the reaction and was assessed by measuring the surface temperature at the bottom of the reaction vessel by means of an infrared sensor. In all cases capped vessels were used, allowing a pressure to build-up. The pressure was evaluated by measuring the bulging of the septum, but never exceeded 20 bars. Tetrahydrofuran (THF) was dried and distilled from sodium benzophenone prior to use. PE was distilled prior to use. Benzonitrile (**102a**), isobutyronitrile (**102d**) and benzaldehyde (**103c**) were dried, distilled, and stored under a dry nitrogen atmosphere. Other commercially available chemicals were used as purchased.

3.5.2 Synthetic procedures and physical data



Diethyl methylphosphonate (**101a**)

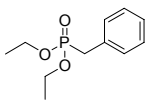
Triethyl phosphite (3.0 mL, 17.5 mmol) and methyl iodide (1.95 mL, 31.3 mmol) were heated in a microwave oven (Power max: 50 W, ramp time: 10 min, hold time 5 min at 130°C). The ethyl iodide formed as a side product was distilled off at 40°C and 10 mbar. Yield: 97 %; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.34 (t, *J*=7.0 Hz, 6H; CH_3CH_2), 1.48 (d, *J*=17.5 Hz, 3H; CH_3), 4.04–4.10 (m, 4H; CH_2); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=10.4 (d, *J*=144.2 Hz), 15.7 (d, *J*=6.2 Hz), 60.6 (d, *J*=6.2 Hz); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=29.7.[29]



Diethylethylphosphonate (**101b**)

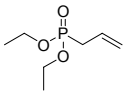
Triethyl phosphite (1.0 mL, 5.83 mmol) and ethyl bromide (0.44 mL, 5.83 mmol) were heated in a microwave oven (Power max. 50 W, ramp time 10 min, hold time 15 min at 200°C and 10 min at 180°C). Ethyl bromide was distilled off from the resulting solution at 40°C and 100 mbar. Yield: 100%; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=0.83 (dt, *J*=19.9 Hz,

J=7.7 Hz, 3H; C*H*$_3$CH$_2$P), 1.00 (t, *J*=7.1 Hz, 6H; C*H*$_3$CH$_2$O), 1.32–1.49 (m, 2H; C*H*$_2$P), 3.70–3.83 (m, 4H; C*H*$_2$O); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=6.3 (d, *J*=6.8 Hz), 16.2 (d, *J*=6.0 Hz), 18.5 (d, *J*=142.6 Hz), 61.1 (d, *J*=6.5 Hz); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=34.4.[29]



Diethylbenzylphosphonate (**101c**)

Triethyl phosphite (3.0 mL, 17.5 mmol) and benzyl bromide (2.71 mL, 22.75 mmol) were heated in a microwave oven (Power max. 200 W, ramp. time 10 min, hold time 15 min at 140°C). Ethyl bromide (40°C and 100 mbar) and diethylethylphosphonate (90°C and 20 mbar) were distilled off to leave the pure product. Yield: 98 %; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.26 (t, *J*=7.0 Hz, 6H; C*H*$_3$), 3.18 (d, *J*=21.7 Hz, 2H; C*H*$_2$Ph), 3.97–4.10 (m, 4H; C*H*$_2$), 7.25–7.34 (m, 5H; Ph-*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=16.1 (d, *J*=6.0 Hz), 33.5 (d, *J*=138.0 Hz), 61.9 (d, *J*=6.8 Hz), 126.6 (d, *J*=3.6 Hz), 128.3 (d, *J*=3.1 Hz; 2C), 129.6 (d, *J*=6.5 Hz, 2C), 131.4 (d, *J*=9.1 Hz); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=27.6.[29]

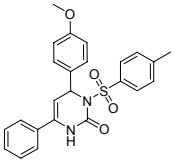


Diethylallylphosphonate (**101d**)

Triethyl phosphite (1.0 mL, 5.83 mmol) and allylbromide (0.6 mL, 6.93 mmol) were heated in a microwave oven (Power max. 250 W, ramp time 10 min, hold time 5 min at 180°C). Ethyl bromide was distilled off from the resulting solution at 40°C and 100 mbar. Yield: 94 %; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.35 (t, *J*=7.1 Hz, 6H; C*H*$_3$), 2.64 (dd, *J*=7.4 Hz, *J*=21.9 Hz, 2H; PC*H*$_2$), 4.08-4.20 (m, 4H; C*H*$_2$CH$_3$), 5.22-5.29 (m, 2H; C*H*$_2$=CH), 5.77-5.88 (m, 1H; C*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ= 16.0 (d, *J*=6.0 Hz), 31.4 (d, *J*=139.3 Hz), 61.5 (d, *J*=6.6 Hz), 119.5 (d, *J*=14.4 Hz), 127.2 (d, *J*=11.2 Hz); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=27.9. [29]

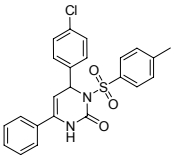
General procedure for the synthesis of 106, **107**, **108**, **114**, **116 and 118 via the HWE/aza-DA reaction**.

*n*BuLi (1.6 M in hexane; 1.2 equiv) was added dropwise to a stirred solution of phosphonate **101** (0.2 M in dry THF) at –78°C. The resulting solution was stirred for 1.5 h and then nitrile **102** (1.1 equiv) was added. The reaction mixture was allowed to warm to –5°C over 1.5 h and then aldehyde **103** (1.1 equiv) was added. The mixture was stirred for a further 0.5 h at –5°C and thereafter for 1.5 h at room temperature. Isocyanate **105** (1.1 equiv) was then added dropwise over 10 min and the resulting solution was stirred overnight. The solvent was removed under reduced pressure and the crude product was purified by crystallisation, column chromatography (PE/EA 4/1 → 1/1) or column chromatography and recrystallisation.



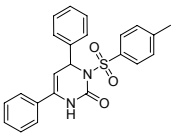
4-(4-methoxyphenyl)-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (**106a**)

The product was crystallised from THF. Yield: 65%; m.p.179–182°C [THF]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=2.32 (s, 3H; C*H*$_3$Ph), 3.83 (s, 3H; C*H*$_3$O), 5.51 (d, *J*=6.1 Hz, 1H; C*H*C), 6.15 (d, *J*=6.1 Hz, 1H; C*H*N), 6.88 (d, *J*=8.6 Hz, 2H; Ph-*H*), 6.99 (d, *J*=8.3 Hz, 2H; Ph-*H*), 7.34–7.53 (m, 9H; Ph-*H*), 7.93 (s, 1H; N*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=21.4, 55.3, 59.1, 101.7, 114.2 (2C), 125.2 (2C), 125.3 (2C), 128.5 (2C), 128.7 (2C), 128.9 (2C), 129.3, 132.9 (2C), 134.0, 136.0, 143.9, 150.5, 159.6; IR (KBr): $\tilde{\nu}$=3243 (m), 3125 (m), 2932 (m), 1691 (s), 1356 (s), 1248 (s), 1168 (s); HRMS (EI): *m/z*=434.1285 $[M]^+$, calc. for $C_{24}H_{22}N_2O_4S$=434.1300.



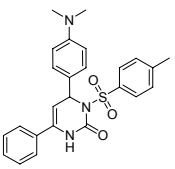
4-(4-chlorophenyl)-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106b)

The product was crystallised from THF. Yield: 80%; m.p. 186–189°C [THF]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=2.35 (s, 3H; C*H*$_3$Ph), 5.39 (d, *J*=6.1 Hz, 1H; C*H*C), 6.05 (d, *J*=6.1 Hz, 1H; C*H*N), 7.00 (d, *J*=8.5 Hz, 2H; Ph-*H*), 7.24–7.45 (m, 12H; Ph-*H* and N*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ= 21.4, 58.8, 100.8, 125.2, 128.5 (2C), 128.7 (2C), 128.9 (6C), 129.5 (2C), 132.6, 134.2, 134.6, 135.7, 139.2, 144.4, 150.3; IR (KBr): $\tilde{\nu}$=3239 (m), 3122 (m), 1693 (s), 1681 (s), 1360 (s), 1171 (s); HRMS (EI): *m/z*=438.0809 $[M]^+$, calc. for $C_{23}H_{19}ClN_2O_3S$=438.0805.



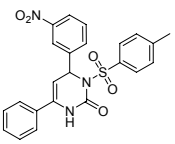
4,6-diphenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106c)

The product was purified by column chromatography and recrystallised from THF/pentane. Yield: 71%; m.p.183–185°C [THF]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=2.33 (s, 3H; C*H*$_3$Ph), 5.52 (d, *J*=6.1 Hz, 1H; C*H*C), 6.17 (d, *J*=6.1 Hz, 1H; C*H*N), 7.00 (d, *J*=8.2 Hz, 2H; Ph-*H*), 7.29–7.48 (m, 12H; Ph-*H*), 7.65 (s, 1H; N*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=21.4, 59.6, 101.4, 125.2 (2C), 127.2 (2C), 128.4, 128.5 (2C), 128.8 (2C), 128.9 (4C), 129.4, 132.8, 134.3, 135.8, 140.7, 144.0, 150.5; IR (KBr): $\tilde{\nu}$=3242 (m), 2986 (m), 1696 (s), 1683 (s), 1395 (s), 1361 (s), 1169 (s) 1086 (s); HRMS (EI): *m/z*=404.1193 $[M]^+$, calc. for $C_{23}H_{20}N_2O_3S$=404.1195.



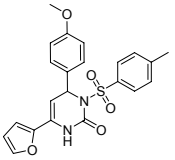
4-(4-(dimethylamino)phenyl)-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106d)

The product was purified by column chromatography. Yield: 54%; m.p. 187–189°C [EA]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=2.28 (s, 3H; C*H*$_3$Ph), 2.94 (s, 6H; (C*H*$_3$)$_2$N), 5.45 (d, *J*=6.1 Hz, 1H; C*H*C), 6.03 (d, *J*=6.1 Hz, 1H; C*H*N), 6.68 (d, *J*=8.8 Hz, 2H; Ph-*H*), 7.00 (d, *J*=8.2 Hz, 2H; Ph-*H*), 7.28–7.40 (m, 10H; Ph-*H* and N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=21.4, 40.6 (2C), 59.5, 102.3, 112.5 (2C), 125.3 (2C), 128.4, 128.6 (4C), 129.0 (2C), 129.1 (2C), 129.3, 133.3, 133.8, 136.5, 143.7, 150.6, 150.8; IR (KBr): $\tilde{\nu}$=3208 (m), 3108 (m), 2926 (m), 1674 (s), 1522 (s), 1350 (s), 1169 (s); HRMS (EI): *m/z*=447.1621 $[M]^+$, calc. for $C_{25}H_{25}N_3O_3S$=447.1617.



4-(3-nitrophenyl)-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106e)

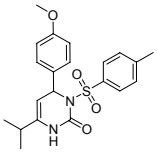
The product was purified by column chromatography. Yield: 38%; m.p. 195–197°C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=2.30 (s, 3H; C*H*$_3$Ph), 5.47 (d, *J*=6.1 Hz, 1H; C*H*C), 6.27 (d, *J*=6.1 Hz, 1H; C*H*N), 7.07 (d, *J*=8.4 Hz, 2H; *m*-C*H* (Ts)), 7.42–7.54 (m, 7H; Ph-*H*), 7.73 (d, *J*=7.8 Hz, 1H; Ph-*H*), 8.00 (s, 1H, N*H*), 8.14–8.17 (m, 2H; Ph-*H*)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=21.6, 59.7, 99.9, 121.9, 123.3, 125.3 (2C), 129.0 (4C), 129.2 (2C), 130.0, 130.1, 132.5, 133.0, 135.7, 135.8, 142.9, 145.0, 148.6, 149.8; IR (KBr): $\tilde{\nu}$=3217 (m), 3106 (m), 1694 (s), 1676 (s), 1528 (s), 1348 (s), 1169 (s); HRMS (EI): *m/z*=449.1064 $[M]^+$, calc. for $C_{23}H_{19}N_3O_5S$=449.1045.



6-(furan-2-yl)-4-(4-methoxyphenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106f)

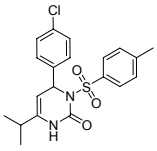
The product was crystallised from DEE. Yield: 61%; m.p. 114°C (decomp) [DEE]; ^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=2.26 (s, 3H; C*H*$_3$Ph), 3.74 (s, 3H; C*H*$_3$O), 5.56 (dd, *J*=1.2 Hz, *J*=6.1 Hz, 1H; C*H*C), 6.04 (d, *J*=6.1 Hz, 1H; C*H*N), 6.38 (dd, *J*=1.6 Hz, *J*=3.2 Hz, 1H; C*H* (Furan)), 6.58 (d, *J*=3.2 Hz, 1H; C*H* (Furan)), 6.77 (d, *J*=8.6

Hz, 2H; *m*-C*H* (PhOMe)), 6.99 (d, *J*=8.1 Hz, 2H; *m*-C*H* (Ts)), 7.25 (d, *J*=8.6 Hz, 2H; *o*-C*H* (PhOMe)), 7.32–7.34 (m, 3H; C*H* (Furan) and *o*-C*H* (Ts)), 7.71 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=21.9, 55.8, 59.5, 100.0, 107.7, 112.1, 114.7 (2C), 126.1, 129.2 (2C), 129.3 (4C), 133.3, 136.7, 143.4, 144.6, 146.5, 150.8, 160.2; IR (KBr): $\tilde{\nu}$=3243 (w), 3121 (w), 1697 (s), 1684 (s), 1510 (m), 1169 (s); HRMS (EI): *m/z*=424.1118 $[M]^+$, calc. for $C_{22}H_{20}N_2O_5S$=424.1093.



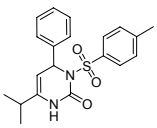
6-isopropyl-4-(4-methoxyphenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106g)

The product was crystallised from THF. Yield: 73%; m.p. 178–179°C [THF]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.01 (d, *J*=6.9 Hz, 6H; $(CH_3)_2CH$), 2.15-2.26 (m, 1H; C*H*$(CH_3)_2$), 2.26 (s, 3H; CH_3Ph), 3.76 (s, 3H; CH_3O), 4.84 (d, *J*=5.7 Hz, 1H; C*H*C), 5.83 (d, *J*=5.7 Hz, 1H; C*H*N), 6.82 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.01 (d, *J*=8.4 Hz, 2H; Ph-*H*), 7.22–7.30 (m, 4H; Ph-*H*), 7.61 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=19.1, 19.2, 20.5, 29.9, 54.4, 58.1, 97.7, 113.1 (2C), 127.6 (2C), 127.8 (2C), 127.9 (2C), 132.8, 135.6, 138.9, 142.9, 149.8, 158.7; IR (KBr): $\tilde{\nu}$=3223 (m), 3111 (m), 2969 (m), 1676 (s), 1510 (s), 1343 (s), 1169 (s); HRMS (EI): *m/z*=400.1444 $[M]^+$, calc. for $C_{21}H_{24}N_2O_4S$=400.1457.



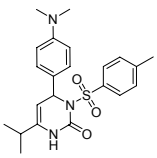
4-(4-chlorophenyl)-6-isopropyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106h)

The product was crystallised from THF. Yield: 65%; m.p. 186–187°C [THF]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.08 (d, *J*=6.8 Hz, 6H; $(CH_3)_2CH$), 2.20–2.30 (m, 1H; C*H*$(CH_3)_2$), 2.33 (s, 3H; CH_3Ph), 4.87 (d, *J*=5.3 Hz, 1H; C*H*C), 5.9 (d, *J*=5.3 Hz, 1H; C*H*N), 7.03–7.35 (m, 8H; Ph-*H*), 7.90 (s, 1H; N*H*); ^{13}C MR (100.61 MHz, $CDCl_3$): δ=20.1 (2C), 21.5, 30.6, 58.8, 97.9, 128.7 (4C), 128.9 (2C), 129.0 (2C), 134.1, 136.3, 140.2, 140.7, 144.3, 155.0; IR (KBr): $\tilde{\nu}$=3225 (m), 3117 (m), 2962 (m), 1705 (s), 1676 (s), 1344 (s), 1169 (s); HRMS (EI): *m/z*=404.0961 $[M]^+$, calc. for $C_{20}H_{21}ClN_2O_3S$=404.0961.



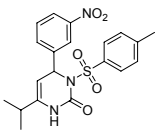
6-isopropyl-4-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106i)

The product was purified by column chromatography. Yield: 55%; m.p. 178–180°C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.05 (d, *J*=6.8 Hz, 6H; $(CH_3)_2CH$), 2.15–2.30 (m, 1H; C*H*$(CH_3)_2$), 2.29 (s, 3H; CH_3Ph), 4.91 (d, *J*=5.7 Hz, 1H; C*H*C), 5.91 (d, *J*=5.7 Hz, 1H; C*H*N), 6.98 (d, *J*=8.3 Hz, 2H; Ph-*H*), 7.21–7.30 (m, 8H; Ph-*H*), 7.97 (s, 1H; N*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=19.9, 20.0, 21.4, 30.8, 59.5, 98.2, 127.2 (2C), 128.2, 128.4 (2C), 128.7 (2C), 128.8 (2C), 136.2, 140.1, 141.5, 143.8, 151.1; IR (KBr): $\tilde{\nu}$=3219 (m), 3107 (m), 2962 (m), 1705 (s), 1678 (s), 1356 (s), 1169 (s); HRMS (EI): *m/z*=370.1358 $[M]^+$, calc. for $C_{20}H_{22}N_2O_3S$=370.1351.



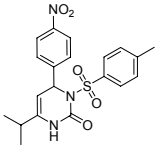
4-(4-(dimethylamino)phenyl)-6-isopropyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106j)

The product was crystallised from THF. Yield: 49%; m.p. 178–180°C [THF]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.05 (d, *J*=6.8 Hz, 6H; $(CH_3)_2CH$), 2.20–2.29 (m, 1H; C*H*$(CH_3)_2$), 2.29 (s, 3H; CH_3Ph), 2.94 (s, 6H; $(CH_3)_2$N), 4.88 (d, *J*=5.7 Hz, 1H; C*H*C), 5.83 (d, *J*=5.7 Hz, 1H; C*H*N), 6.62 (d, *J*=8.8 Hz, 2H; Ph-*H*), 6.99 (d, *J*=8.2 Hz, 2H; Ph-*H*), 7.15–7.29 (m, 4H; Ph-*H*) 7.40 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=20.6 (2C), 21.9, 31.0, 41.0 (2C), 59.8, 99.3, 112.9 (2C), 128.9 (2C), 129.0 (2C), 129.3 (2C), 129.6, 137.2, 139.9, 143.9, 151.1, 151.3; IR (KBr): $\tilde{\nu}$=3230 (m), 2971 (m), 1676 (s), 1350 (s), 1169 (s); HRMS (EI): *m/z*=413.1755 $[M]^+$, calc. for $C_{22}H_{27}N_3O_3S$=413.1773.



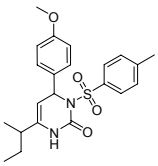
6-isopropyl-4-(3-nitrophenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106k)

The product was purified by column chromatography. Yield: 60%; m.p. 155–157°C [EA]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.14 (d, *J*=6.1 Hz, 6H; (CH_3)$_2$CH), 2.25–2.35 (m, 1H; C*H*(CH$_3$)$_2$), 2.33 (s, 3H; C*H*$_3$Ph), 4.97 (d, *J*=5.3 Hz, 1H; C*H*C), 6.11 (d, *J*=5.3 Hz, 1H; C*H*N), 7.07 (d, *J*=8.0 Hz, 2H; *m*-C*H* (Ts)), 7.49–7.57 (m, 4H; Ph-*H*), 7.67 (d, *J*=7.7 Hz, 1H; C*H* (3-NO$_2$Ph)), 8.14–8.18 (m, 2H; Ph-*H* and N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=20.1 (2C), 21.5, 30.7, 58.5, 96.9, 122.0, 123.1, 128.8 (2C), 128.9 (2C), 130.0, 133.1, 136.0, 141.9, 143.6, 144.7, 148.5, 150.7; IR (KBr): *ν̃*=3243 (m), 2967 (m), 1676 (s), 1535 (s), 1356 (s), 1169 (s), 689 (m), 573 (m); HRMS (EI): *m/z*=415.1223 [M]$^+$, calc. for $C_{20}H_{21}N_3O_5S$=415.1202.



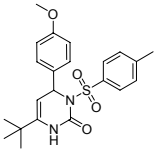
6-isopropyl-4-(4-nitrophenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106l)

The product was purified by column chromatography and recrystallised from THF/pentane. Yield: 55%; m.p. 170–171°C [THF]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.07 (d, *J*=6.5 Hz, 6H; (CH_3)$_2$CH), 2.11–2.28 (m, 1H; C*H*(CH$_3$)$_2$), 2.35 (s, 3H; C*H*$_3$Ph), 4.91 (d, *J*=5.4 Hz, 1H; C*H*C), 6.05 (d, *J*=5.4 Hz, 1H; C*H*N), 6.91 (s, 1H; N*H*), 7.10 (d, *J*=8.2 Hz, 2H; *m*-C*H* (Ts)), 7.44–7.47 (m, 4H; *o*-C*H* (Ts) and *o*-C*H* (4-NO$_2$Ph)), 8.16 (d, *J*=8.2 Hz, 2H; *m*-C*H* (4-NO$_2$Ph)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=20.0, 20.1, 21.6, 30.7, 58.7, 97.0, 124.2 (2C), 127.7 (2C), 128.9 (4C), 135.9, 141.4, 144.9, 147.7, 148.4, 150.2; IR (KBr): *ν̃*=3211 (w), 3113 (w), 2963 (w), 1709 (m), 1680 (s), 1522 (s), 1346 (s), 1171 (s); HRMS (EI): *m/z*=415.1218 [M]$^+$, calc. for $C_{20}H_{21}N_3O_5S$=415.1202.



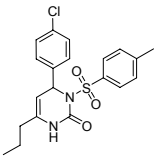
6-sec-butyl-4-(4-methoxyphenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106m)

The product was crystallised from THF and was thereby obtained as a 1:1 mixture of diastereomers. Yield: 49%; m.p. 170–171°C [THF]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ= 0.78 (t, *J*=8.0 Hz, 6H; C*H*$_3$CH), 1.09 (d, *J*=8.2 Hz, 6H; C*H*$_3$CH), 1.18–1.49 (m, 4H; C*H*$_2$CH), 2.01–2.17 (m, 2H; C*H*CH$_3$), 2.36 (s, 6H; C*H*$_3$Ph), 3.73 (s, 6H; C*H*$_3$O), 4.90 (d, *J*=5.8 Hz, 2H; C*H*C), 5.87 (dd, *J*=5.8 Hz, *J*=3.2 Hz, 2H; C*H*N), 6.53 (s, 2H; N*H*), 6.82 (d, *J*=8.7 Hz, 4H; *o*-C*H* (PhOMe)), 7.09 (d, *J*=8.4 Hz, 4H; *m*-C*H* (Ts)), 7.28–7.41 (m, 8H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=10.8 (2C), 17.1, 17.4, 20.8 (2C), 26.1, 26.3, 36.8, 36.9, 54.7 (2C), 58.3, 58.4, 98.3, 98.6, 113.3 (4C), 127.9 (4C), 128.0 (4C), 128.2 (4C), 133.3, 133.4, 136.0 (2C), 138.6 (2C), 143.1 (2C), 149.6 (2C), 158.8 (2C); IR (KBr): *ν̃*=3239 (m), 2962 (m), 1681 (s), 1509 (m), 1345 (s), 1167 (s); HRMS (EI): *m/z*=414.1603 [M]$^+$, calc. for $C_{22}H_{26}N_2O_4S$=414.1613.



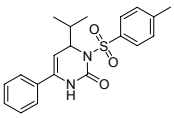
6-tert-butyl-4-(4-methoxyphenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106n)

The product was purified by column chromatography. Yield: 16%; m.p. 197–202°C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.10 (s, 9H; (CH_3)$_3$C), 2.31 (s, 3H; C*H*$_3$Ph), 3.81 (s, 3H; C*H*$_3$O), 4.95 (d, *J*=5.9 Hz, 1H; C*H*C), 5.89 (d, *J*=5.9 Hz, 1H; C*H*N), 6.82 (d, *J*=8.7 Hz, 2H; *o*-C*H* (PhOMe)), 7.03 (d, *J*=8.2 Hz, 2H; *m*-C*H* (Ts)), 7.15–7.26 (m, 5H, Ph-*H* and N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=21.5, 27.7 (3C), 33.2, 55.4, 58.9, 98.4, 114.1 (2C), 128.6 (2C), 128.8 (4C), 133.7, 136.5, 142.0, 143.8, 150.8, 159.7; IR (KBr): *ν̃*=3435 (m), 3237 (w), 2961 (m), 1676 (s), 1350 (s), 1260 (s), 1163 (s); HRMS (EI): *m/z*=414.1613 [M]$^+$, calc. for $C_{22}H_{26}N_2O_4S$=414.1613.



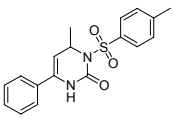
4-(4-chlorophenyl)-6-propyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106o)

The product was purified by column chormatography; Yield: < 5%; m.p. 144–147°C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=0.90 (t, *J*=7.3 Hz, 3H; C*H*$_3$CH$_2$), 1.43–1.57 (m, 2H; CH$_2$*C*H$_3$), 2.03 (t, *J*=7.4 Hz, 2H; C*H*$_2$C), 2.34 (s, 3H; C*H*$_3$Ph), 4.90 (d, *J*=5.8 Hz, 1H; C*H*C), 5.90 (d, *J*=5.8 Hz, 1H; C*H*N), 7.05–7.39 (m, 9H; Ph-*H* and N*H*); ^{13}C NMR (62.90 MHz, $CDCl_3$): δ=13.3, 19.7, 21.5, 33.8, 59.0, 100.2, 127.8 (2C), 128.5 (2C), 128.7 (2C), 128.9 (2C), 134.1, 134.8, 136.2, 140.1, 144.4, 150.4; IR (KBr): $\tilde{\nu}$=3223 (m), 3113 (m), 2961 (m), 1709 (s), 1680 (s), 1356 (s), 1167 (s), 1088 (s); HRMS (EI): *m/z*=404.0940 [M]$^+$, calc. for $C_{20}H_{21}ClN_2O_3S$=404.0961.



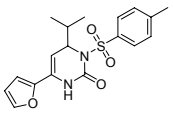
4-isopropyl-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106p)

The product was purified by column chromatography. Yield: 35%; m.p. 170–171°C [THF]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.88 (d, *J*=6.8 Hz, 3H; C*H*$_3$CH), 0.96 (d, *J*=6.8 Hz, 3H; C*H*$_3$CH), 2.18–2.31 (m, 1H; C*H*(CH$_3$)$_2$), 2.34 (s, 3H; C*H*$_3$Ph), 4.99–5.02 (m, 1H; CHC), 5.23 (dd, *J*=6.1 Hz, *J*=1.4 Hz, 1H; C*H*N), 6.68 (s, 1H; N*H*), 7.32 (s, 3H; Ph-*H*), 7.82–7.84 (m, 4H; Ph-*H*), 7.83 (d, *J*=8.2 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=16.1, 18.7, 22.0, 35.7, 61.9, 98.2, 125.6 (2C), 129.3 (2C), 129.5 (2C), 129.6 (2C), 129.9, 133.7, 136.9, 137.0, 144.8, 151.2; IR (KBr): $\tilde{\nu}$=3238 (m), 2965 (m), 1693 (s), 1674 (s), 1344 (s), 1171 (s); HRMS (EI): *m/z*=369.1280 [M]$^+$, calc. for $C_{20}H_{22}N_2O_3S$=369.1273.



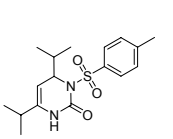
4-methyl-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106q)

The product was purified by column chromatography and recrystallised from THF/pentane. Yield: 15%; m.p. 177–179°C [THF]; ^{1}H NMR: (200.13 MHz, $CDCl_3$): δ=1.45 (d, *J*=6.5 Hz, 3H; C*H*$_3$CHN), 2.40 (s, 3H; C*H*$_3$Ph), 5.21–5.35 (m, 1H; C*H*CH$_3$), 5.40 (dd, *J*=6.2 Hz, *J*=1.9 Hz, 1H; C*H*C), 6.89 (s, 1H; N*H*), 7.26 (d, *J*=8.2 Hz, 2H; *o*-C*H* (Ts)), 7.38 (s, 5H; Ph-*H*), 7.93 (d, *J*=8.2 Hz, 2H; *m*-C*H* (Ts)); ^{13}C NMR: (250.13 MHz, $CDCl_3$): δ=22.0, 22.7, 52.6, 102.9, 125.5 (2C), 129.3 (2C), 129.4 (2C), 129.6 (2C), 129.9, 133.5, 135.7, 137.1, 144.9, 150.6; IR (KBr): $\tilde{\nu}$=3236 (m), 3116 (m), 1693 (s), 1678 (s), 1409 (s), 1345 (s), 1162 (s); HRMS: (EI): *m/z*=342.1062 [M]$^+$, calc. for $C_{18}H_{18}N_2O_3S$=342.1038.



6-(furan-2-yl)-4-isopropyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106r)

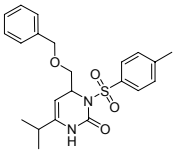
The product was purified by column chromatography. Yield: 36%; m.p. 90°C (decomp.) [EA]; ^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=0.92 (d, *J*=6.8 Hz, 3H; C*H*$_3$CH), 1.01 (d, *J*=6.9 Hz, 3H; C*H*$_3$CH), 2.24–2.29 (m, 1H; C*H*(CH$_3$)$_2$), 2.39 (s, 3H; C*H*$_3$Ph), 5.05–5.09 (m, 1H; C*H*N), 5.47 (dd, *J*=6.2 Hz, *J*=1.9 Hz, 1H; C*H*C), 6.44 (dd, *J*=1.6 Hz, *J*=3.5 Hz, 1H; C*H*CHO (furan)), 6.51 (d, *J*=3.5 Hz, 1H; C*H*C (furan)), 7.08 (s, 1H; *NH*), 7.26 (d, *J*=8.1 Hz, 2H; *m*-C*H* (Ts)), 7.40 (d, *J*=1.6 Hz, 1H; C*H*O (furan)), 7.88 (d, *J*=8.1 Hz, 2H; *o*-C*H* (Ts)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=15.7, 18.2, 21.5, 35.3, 61.4, 95.6, 106.6, 111.6, 127.6, 128.7 (2C), 129.2 (2C), 136.5, 142.8, 144.4, 146.1, 150.5; IR: $\tilde{\nu}$=3243 (w), 2967 (w), 1696 (s), 1684 (s), 1177 (s); HRMS: *m/z*=360.1184 [M]$^+$, calc. for $C_{18}H_{20}N_2O_4S$=360.1143.



4,6-diisopropyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106s)

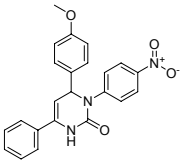
The product was crystallised from THF. Yield: 40%; m.p. 177–181°C [THF]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.82 (d, *J*=6.9 Hz, 3H; C*H*$_3$CH), 0.91 (d, *J*=6.9 Hz, 3H; C*H*$_3$CH), 1.04 (d, *J*=6.8 Hz, 6H; C*H*$_3$CH), 2.08–2.25 (m, 2H; C*H*(CH$_3$)$_2$), 4.70 (d, *J*=5.7 Hz, 1H; C*H*C), 4.87 (t, *J*=5.7 Hz, 1H; C*H*N), 6.87 (s, 1H; N*H*), 7.24 (d, *J*=8.1 Hz, 2H;

m-C*H* (Ts)), 7.85 (d, *J*=8.1 Hz, 2H; *o*-C*H* (Ts)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=15.7, 18.5, 20.7, 20.9, 22.0, 31.1, 35.3, 61.5, 94.0, 129.1 (2C), 129.5 (2C), 137.4, 142.8, 144.6, 151.8; IR (KBr): $\tilde{\nu}$=3455 (w), 2961 (w), 1676 (s), 1350 (m), 1163 (m); HRMS (EI): *m/z*=336.1516 $[M]^+$, calc. for $C_{17}H_{24}N_2O_3S$=336.1508.



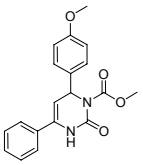
4-(benzyloxymethyl)-6-isopropyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106t)

The product was purified by column chromatography. Yield: 21%; m.p. 148–149°C [EA]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.09 (d, *J*=6.9 Hz, 6H; (CH_3)$_2$CH), 2.14–2.29 (m, 1H; C*H*(CH$_3$)$_2$), 2.42 (s, 3H; CH_3Ph), 3.42–3.53 (m, 2H; CH_2CH), 4.51 (s, 2H; CH_2Ph), 4.87 (d, *J*=5.2 Hz, 1H; C*H*C), 5.11–5.19 (m, 1H; C*H*N), 6.45 (s, 1H; N*H*), 7.22–7.98 (m, 9H; Ph-*H*); ^{13}C NMR (62.90 MHz, $CDCl_3$): δ=20.2 (2C), 21.6, 30.6, 55.1, 72.0, 73.1, 95.1, 127.4 (2C), 127.6, 128.0 (2C), 128.9 (2C), 129.0 (2C), 136.9, 138.0, 143.1, 144.2, 150.8; IR (KBr): $\tilde{\nu}$=3221 (w), 3115 (w), 2963 (w), 1699 (s), 1670 (s), 1348 (s), 1167 (s); HRMS (EI): *m/z*=414.1612 $[M]^+$, calc. for $C_{22}H_{26}N_2O_4S$=414.1613.



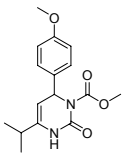
4-(4-methoxyphenyl)-3-(4-nitrophenyl)-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (106u)

The product was crystallised from THF/pentane. Yield: 26%; m.p. 211–214°C [THF]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=3.77 (s, 3H; CH_3O), 5.34 (d, *J*=5.1 Hz, 1H; C*H*C), 5.45 (d, *J*=5.1 Hz, 1H; C*H*N), 6.79–8.14 (m, 14H; Ph-*H* and N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=55.3, 63.8, 100.1, 114.5 (2C), 124.2 (2C), 125.1 (2C), 127.4 (2C), 127.8 (2C), 129.0 (2C), 129.5, 133.1, 133.6, 134.7, 145.4, 147.1, 152.4, 159.6; IR (KBr): $\tilde{\nu}$=3225 (w), 3109 (w), 1667 (s), 1510 (s), 1345 (s); HRMS (EI): *m/z*=401.1387 $[M]^+$, calc. for $C_{23}H_{19}N_3O_4$=401.1376.



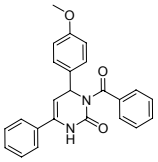
Methyl-6-(4-methoxyphenyl)-2-oxo-4-phenyl-2,3-dihydropyrimidine-1(6*H*)-carboxylate (106v)

The product was purified by column chromatography. Yield: 70%; m.p: 147–150°C [EA]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=3.68 (s, 3H; CH_3), 3.80 (s, 3H; CH_3), 5.49 (dd, *J*=6.4 Hz, *J*=1.5 Hz, 1H; C*H*C), 5.87 (d, *J*=6.4 Hz, 1H; C*H*N), 6.80 (d, *J*=8.6 Hz, 2H; Ph-*H*), 7.00 (s, 1H; N*H*), 7.29–7.41 (m, 7H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=54.6, 55.7, 57.2, 102.2, 114.6 (2C), 125.6 (2C), 128.6 (2C), 129.5 (2C), 129.9, 132.6, 133.6, 135.6, 150.7, 155.0, 159.9; IR (KBr): $\tilde{\nu}$=3259 (m), 2910 (m), 1734 (s), 1694 (s), 1408 (s), 1268 (s); HRMS: *m/z*=338.1251 $[M]^+$, calc. for $C_{19}H_{18}N_2O_4$=338.1267.



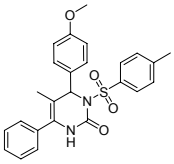
Methyl 4-isopropyl-6-(4-methoxyphenyl)-2-oxo-2,3-dihydropyrimidine-1(6H)-carboxylate (106w)

The product was purified by column chromatography and isolated as a light-yellow oil. Yield: 29%; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.06 (d, *J*=6.9 Hz, 6H; (CH_3)$_2$CH), 2.20–2.36 (m, 1H; C*H*(CH$_3$)$_2$), 3.71 (s, 3H; CH_3), 3.76 (s, 3H; COOCH_3), 4.92 (d, *J*=6.1 Hz, 1H; C*H*C), 5.65 (d, *J*=6.1 Hz, 1H; C*H*N), 6.76 (d, *J*=8.8 Hz, 2H; *m*-C*H* (PhOMe)), 7.22 (d, *J*=8.8 Hz, 2H; *o*-C*H* (PhOMe)), 7.58 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=20.4, 20.8, 20.9, 54.3, 55.6, 57.0, 99.0, 114.5 (2C), 128.5 (2C), 133.4, 141.7, 151.7, 155.1, 159.7; IR (KBr): $\tilde{\nu}$=3240 (w), 2962 (w), 1770 (s), 1714 (s), 1512 (m), 1255 (s); HRMS: *m/z*=304.1433 $[M]^+$, calc. for $C_{16}H_{20}N_2O_4$=304.1423.



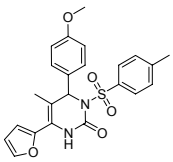
3-benzoyl-4-(4-methoxyphenyl)-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (106x)

The product was crystallised from THF. Yield: 70%; m.p. 186–187°C (decomp) [THF]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=3.78 (s, 3H; C*H*$_3$), 5.74 (d, *J*=6.3 Hz, 1H; C*H*C), 6.01 (d, *J*=6.3 Hz, 1H; C*H*N), 6.87 (d, *J*=8.8 Hz, 2H; *o*-C*H* (PhOMe)), 7.35–7.46 (m, 10H; Ph-*H*), 7.57–7.59 (m, 2H; *o*-C*H* (COPh)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=55.3, 55.9, 102.9, 114.1 (2C), 125.1 (2C), 127.8 (2C), 128.0 (2C). 128.4 (2C), 129.1 (2C), 129.5, 131.2, 132.0, 133.0, 135.4, 136.3, 152.3, 159.4, 171.7; IR (KBr): $\tilde{\nu}$=3282 (s), 2968 (w), 2839 (w), 1702 (s), 1688 (s), 1509 (s), 1396 (s); HRMS (EI): *m/z*=384.1485 $[M]^+$, calc. for $C_{24}H_{20}N_2O_3$=384.1474.



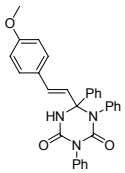
4-(4-Methoxyphenyl)-5-methyl-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106y)

The product was crystallised from ethyl acetate. Yield: 35%; m.p. 194–195°C [EA]; ^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=1.65 (s, 3H; C*H*$_3$C), 2.32 (s, 3H; *CH*$_3$Ph), 3.83 (s, 3H; C*H*$_3$O), 5.75 (s, 1H; C*H*N), 6.28 (s, 1H; N*H*), 6.85 (dd, *J*=6.7 Hz, *J*=2.0 Hz, 2H; *m*-C*H* (Ph)), 7.04 (d, *J*=8.3 Hz, 2H; *m*-C*H* (Ts)), 7.28–7.40 (m, 9H; Ph-*H*); ^{13}C NMR: (100.61 MHz, $CDCl_3$): δ=15.7, 21.5, 55.4, 63.9, 109.8, 114.2 (2C), 128.5 (2C), 128.7 (2C), 128.8, 128.9 (2C), 129.0 (2C), 129.1 (3C), 132.2, 133.8, 136.4, 144.0, 149.6, 159.9; IR (KBr): $\tilde{\nu}$=3208 (m), 3102 (m), 2932 (m), 1680 (s), 1511 (m), 1351 (m), 1167 (s); HRMS (EI): *m/z*=448.1465 $[M]^+$, calc. for $C_{25}H_{24}N_2O_4S$=448.1457.



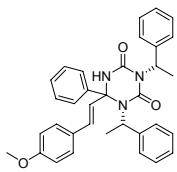
6-(furan-2-yl)-4-(4-methoxyphenyl)-5-methyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (106z)

The product was crystallised from ethyl acetate. Yield: 90%; m.p. 202–204°C (decomp) [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.89 (s, 3H; C*H*$_3$C), 2.32 (s, 3H; C*H*$_3$Ph), 3.81 (s, 3H; C*H*$_3$O), 5.77 (s, 1H; C*H*N), 6.45–6.48 (m, 2H; C*H* (Furan)), 6.78 (s, 1H; N*H*), 6.82 (d, *J*=8.8 Hz, 2H; *m*-C*H* (PhOMe)), 7.06 (d, *J*=8.2 Hz, 2H; *m*-C*H* (Ts)), 7.31 (d, *J*=8.8 Hz, 2H; *o*-C*H* (PhOMe)), 7.39 (d, *J*=8.2 Hz, 2H; *o*-C*H* (Ts)), 7.41–7.45 (m, 1H; C*H*O (furan)); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=16.1, 21.4, 55.2, 64.0, 109.9, 110.0, 111.6, 114.0 (2C), 120.5, 128.6 (2C), 128.8 (2C), 129.0 (2C), 131.5, 136.1, 142.0, 144.0, 145.8, 149.5, 159.7; IR (KBr): $\tilde{\nu}$=3225 (m), 3116 (m), 2925 (w), 1688 (s), 1674 (s), 1510 (s), 1258 (s); HRMS (EI): *m/z*=438.1261 $[M]^+$, calc. for $C_{23}H_{22}N_2O_5S$=438.1249.



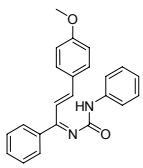
(*E*)-6-(4-methoxystyryl)-1,3,6-triphenyl-1,3,5-triazinane-2,4-dione (107a)

The general HWE/aza-DA procedure was followed using **101a**, **102a**, **103a** and **105c**. The product was purified via column chromatography and afforded 107a as a sticky oil. Yield: 32%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ= 3.82 (s, C*H*$_3$, 3H), 6.25–7.65 (m, Ph-*H* and C=C*H*, 21H); ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ= 55.3, 75.8, 114.3 (2C), 125.6, 127.5 (2C), 127.6, 127.9, 128.2, 128.4 (2C), 128.6 (2C), 128.7 (2C), 128.8 (2C), 129.2 (2C), 129.3, 129.7 (2C), 132.2, 135.0, 137.7, 140.3, 152.6, 153.3, 160.2; IR (KBr): $\tilde{\nu}$=3441 (w), 3088 (w), 1723 (s), 1684 (s), 1516 (s), 1439 (s), 1260 (s); HRMS (EI): *m/z*=475.1893 $[M]^+$, calc. for $C_{30}H_{25}N_3O_3$=475.1896.



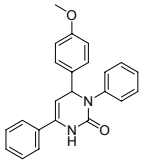
6-(4-methoxystyryl)-6-phenyl-1,3-bis ((*S*)-1-phenylethyl)-1,3,5-triazinane-2,4-dione (**107b**)

The general HWE/aza-DA procedure was followed using **101a**, **102a**, **103a** and (S)-**105f**. The product was purified by column chromatography to afford **107b** in 20% *de*. Yield: 42%; Diastereomeric excess was determined by HPLC analysis (Pathfinder 100PS_5.0μm, methanol/0.02 M ammonium acetate buffer pH=4.5 70/30, 1mL/min); t_r=70.88 min (minor), t_r=63.73 min (major); R=2.22, α=1.12; m.p. 66–69°C [EA]; $[\alpha]^{20}_D$= –72.0 (*c*=1.0 in $CHCl_3$); ^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=1.72 (d, *J*=7.0 Hz, 3H; C*H*$_3$CH), 1.73 (d, *J*=7.0 Hz, 3H; C*H*$_3$CH), 1.75 (d, *J*=7.0 Hz, 3H; C*H*$_3$CH), 1.78 (d, *J*=7.0 Hz, 3H; C*H*$_3$CH), 3.81 (s, 3H; C*H*$_3$O), 3.85 (s, 3H; C*H*$_3$O), 4.44 (q, *J*=7.0 Hz, 1H; CH$_3$C*H*), 4.61 (br s, 1H; CH$_3$C*H*), 5.61–5.66 (m, 2H; CH$_3$C*H*), 6.15 (d, *J*=15.9 Hz, 1H; C*H*CH), 6.29 (d, *J*=15.6 Hz, 1H; C*H*CH), 6.30 (d, *J*=15.9 Hz, 1H; C*H*CH), 6.63 (d, *J*=15.6 Hz, 1H; C*H*CH), 6.81 (d, *J*=8.7 Hz, 2H; Ph-*H*), 6.91 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.00–8.01 (m, 34H; Ph-*H*); ^{13}C NMR: (100.61 MHz, $CDCl_3$): δ=16.5, 16.6, 19.9, 20.2, 51.1 (2C), 55.3, 55.4, 56.4, 57.5, 76.1, 76.6, 114.0 (2C), 114.2 (2C), 125.3, 125.5, 126.2, 126.3, 126.7, 126.9 (4C), 127.0 (2C), 127.1, 127.7 (6C), 127.8 (6C), 128.0 (2C), 128.2 (2C), 128.4 (4C), 128.6 (2C), 129.0 (2C), 129.4, 129.7, 132.3, 132.5, 140.3, 140.5, 141.2 (2C), 142.0, 142.3, 152.2, 152.5, 152.6, 153.1, 160.0, 160.2; IR (KBr): $\tilde{\nu}$=3027 (w), 1711 (s), 1698 (s), 1512 (s), 1446 (s), 1250 (s), 1028 (s); HRMS (EI): *m/z*=531.2522 $[M]^+$, calc. for $C_{34}H_{33}N_3O_3$=531.2522.



1-(3-(4-methoxyphenyl)-1-phenylallylidene)-3-phenylurea (**108a**)

The general HWE/aza-DA procedure was followed using **101a**, **102a**, **103a** and **105c**. After purification by column chromatography **108a** was isolated as sticky oil. Yield: 40%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=3.82 (s, 3H; C*H*$_3$O), 5.19 (s, 1H; N*H*), 6.81–7.72 (m, 16H; Ph-*H* and C*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=55.4, 114.1 (2C), 118.9, 119.0 (2C), 123.8, 127.2, 128.4 (2C), 129.0 (2C), 129.3 (2C), 129.9 (2C), 130.7, 137.3, 137.9, 145.5, 161.1 (2C), 172.3; IR (KBr): $\tilde{\nu}$=3380 (w), 3017 (w), 1580 (s), 1254 (m), 1173 (m); HRMS (EI): *m/z*=356.1523 $[M]^+$, calc. for $C_{23}H_{20}N_2O_2$=356.1525.



4-(4-methoxyphenyl)-3,6-diphenyl-3,4-dihydropyrimidin-2(1H)-one (**109**)

DHPM **109** was prepared under MW conditions from **106b** (procedure A) or from **108a** (procedure B).

Procedure A: DHPM **106b** (23 mg, 0.053 mmol) and phenylisocyanate (1.2 equiv) were dissolved in dry dioxane (1 mL) and heated in a microwave oven (Power max. 300 W, ramp. time 30 min, hold time 60 min at 190°C). The solvent was then evaporated and the crude product was purified by column chromatography using PE/EA (4/1)→ EA. DHPM **109** was isolated in 58% yield.

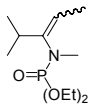
Procedure B: urea **108a** (47 mg, 0.13 mmol) was dissolved in toluene (2.5 mL) and heated in a microwave oven (Power max. 300W, ramp. time 20 min, hold time 75 min at 120°C). The solvent was then evaporated and the crude product was purified by column chromatography using PE/EA (3/1)→ EA. DHPM **109** was isolated in 38% yield. m.p. 139–142°C [EA]; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=3.81 (s, 3H; C*H*$_3$O), 5.28 (dd, *J*=2.1 Hz, *J*=4.9 Hz, 1H; C*H*C), 5.34 (d, *J*=4.9 Hz, 1H; C*H*N), 6.80 (d, *J*=8.7 Hz, 2H; *m*-C*H* (PhOMe)), 6.93 (s, 1H; N*H*), 7.07–7.51 (m, 12H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=55.2, 64.6, 99.9, 114.0 (2C), 125.0 (2C), 127.0, 128.0 (2C), 128.5 (2C), 128.9 (4C), 129.1, 134.1, 134.2, 134.9, 141.0, 153.0, 159.4; IR (NaCl): $\tilde{\nu}$=2902 (w), 2839 (w), 1652 (s), 1507 (s), 1249 (m), 1174 (m); HRMS (EI): *m/z*=356.1516 $[M]^+$, calc. for $C_{23}H_{20}N_2O_2$=356.1525.

General procedure for the trapping experiments with MeI and H_2O

*n*BuLi (1.6 M in hexane; 1.2 equiv) was added dropwise to a stirred solution of phosphonate **101** (0.2 M in dry THF) at –78 °C. The resulting solution was stirred for 1.5 h and then nitrile **102** (1.1 equiv) was added. The reaction mixture was allowed to warm to –5 °C over 1.5 h. After completion or overnight stirring at room temperature MeI or H_2O (1.1 equiv) was added. The solvent was then removed from the reaction mixture under reduced pressure at room temperature. The crude product was purified by column chromatography. The composition of the product was analysed by applying various NMR techniques (see below).

Trapping experiments with MeI

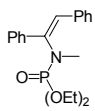
Application of the general procedure (see above) using **101b** and **102b** with overnight stirring gave **110a**.



(*E*,*Z*)-Diethyl N-methyl-N-((Z)-(2-methylpent-3-en-3-yl)) phosphoramidate (110a)

Yield: 37%; *E:Z* = 1:6; (*Z*)-**110a**: ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.02 (d, *J*=6.8 Hz, 6H; (C*H*$_3$)$_2$CH), 1.25–1.29 (m, 6H; C*H*$_3$CH$_2$), 1.60–1.64 (m, 3H; =CHC*H*$_3$), 2.43–2.50 (m, 1H; C*H*(CH$_3$)$_2$), 2.76 (d, *J*=9.0 Hz, 3H; NC*H*$_3$), 3.97–4.13 (m, 4H; OC*H*$_2$CH$_3$), 5.5 (q, *J*=6.8 Hz, 1H; =C*H*); ^{31}P NMR (161.98 MHz, $CDCl_3$): δ=7.2; (*E*)-**110a**: ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.07 (d, *J*=7.0 Hz, 6H; (C*H*$_3$)$_2$CH), 1.25–1.29 (m, 6H; C*H*$_3$CH$_2$), 1.60–1.64 (m, 3H; =CHC*H*$_3$), 2.74 (d, *J*=9.9 Hz, 3H; NC*H*$_3$), 2.78–2.87 (m, 1H; C*H*(CH$_3$)$_2$), 3.97–4.13 (m, 4H; OC*H*$_2$CH$_3$), 5.46 (dq, *J*=2.7 Hz, *J*=7.1 Hz, 1H; =C*H*); ^{31}P NMR (161.98 MHz, $CDCl_3$): δ=7.8. Besides **110a**, 6% of another phosphoramidate was identified.[30]

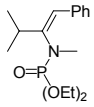
Application of the general procedure (see above) using **101c** and **102a** with overnight stirring gave **110b**.



(*Z*)-Diethyl-1,2-diphenylvinyl-(methyl)phosphoramidate (110b)

Yield: 100%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.24 (t, *J* = 7.1 Hz, 6H; C*H*$_3$CH$_2$), 2.62 (d, *J*=9.1 Hz, 3H; NC*H*$_3$), 4.06–4.18 (m, 4H; C*H*$_2$), 6.49 (s, 1H; =C*H*Ph), 6.82–7.25 (m, 10H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=16.1 (d, *J*=7.2 Hz), 35.5 (d, *J*=5.7 Hz), 64.0 (d, *J*=6.2 Hz), 123.0 (d, *J*=3.2 Hz), 126.5 (s), 127.9 (s, 2C), 128.5 (s, 3C), 129.0 (s, 2C), 129.8 (s, 2C), 135.3 (d, *J*=5.1 Hz), 136.0 (d, *J*=1.2 Hz), 141.0 (d, *J*=3.3 Hz); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=6.0.

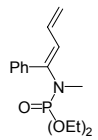
Application of the general procedure (see above) using **101c** and **102b** with overnight stirring gave **110c**.



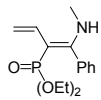
(Z)-diethyl methyl(3-methyl-1-phenylbut-1-en-2-yl)phosphoramidate (110c)

Yield: 20%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.18 (d, *J*=6.8 Hz, 6H; (C*H*$_3$)$_2$CH), 1.28 (td, *J*=7.1 Hz, *J*=0.7 Hz, 6H; C*H*$_3$CH$_2$O), 2.72 (d, *J*=8.8 Hz, 3H; NC*H*$_3$), 2.73–2.81 (m, 1H; C*H*(CH$_3$)$_2$), 3.96–4.14 (m, 4H; C*H*$_2$O), 6.05 (s, 1H; =C*H*Ph), 7.16–7.31 (m, 5H; Ph-*H*); ^{31}P NMR (101.25 MHz, THF): δ=4.0. Besides **101c** and **110c**, 10–20% of another phosphonate was identified.[31]

Application of the general procedure (see above) using **101d** and **102a** with overnight stirring gave **110d** and **113**.



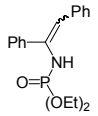
(*E*)-Diethyl N-(methyl)-N-(1-phenylbuta-1,3-dienyl) phosphoramidate (**110d**) (**110d**): Yield: 37%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.16–1.20 (m, 6H; CH_3CH$_2$O), 2.62 (d, *J*=8.9 Hz, 3H; NCH_3), 3.94–4.04 (m, 4H; OCH_2), 4.86 (d, *J*=9.4 Hz, 1H; =CH_2), 5.11 (d, *J*=16.0 Hz, 1H; =CH_2), 6.10–6.38 (m, 2H; =C*H*C*H*=), 7.16–7.53 (m, 5H; Ph-*H*); ^{31}P NMR (101.25 MHz, THF): δ=6.2.



(*E*)-Diethyl 1-(methylamino)-1-phenylbuta-1,3-dien-2-ylphosphonate (**113**): Yield: 63%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.16–1.20 (m, 6H; CH_3CH$_2$O), 2.89 (d, *J*=6.5 Hz, 3H; NCH_3), 3.73–4.04 (m, 4H; OCH_2), 5.35 (d, *J*=6.8 Hz, 1H; =CH_2), 5.62–5.69 (m, 1H; =CH_2), 6.98–7.14 (m, 1H; C*H*=CH$_2$), 7.17–7.53 (m, 5H; Ph-*H*); ^{31}P NMR (101.25 MHz, THF): δ=22.1.

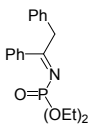
Trapping experiment with H_2O

Application of the general procedure (see above) using **101c** and **102a** with overnight stirring gave **111a** and **112**. Three products were identified (*E*)- and (*Z*)-**111a** (Yield: 58%; *E:Z* = 1:1.5) and **112** (Yield: 42%).

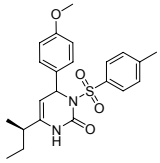


(*Z*)-Diethyl N-1,2-diphenylvinyl)phosphoramidate (**111a**): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.10 (dt, *J*=0.9 Hz, *J*=7.1 Hz, 6H; CH_3), 3.86–3.96 (m, 4H; CH_2), 5.91 (s, 1H; C*H*Ph), 7.16–7.57 (m, 10H; Ph-*H*); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=3.7.

(*E*)-Diethyl N-1,2-diphenylvinyl)phosphoramidate (**111a**): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.32 (dt, *J*=0.7 Hz, *J*=7.1 Hz, 6H; CH_3), 4.11–4.16 (m, 4H; CH_2), 6.32 (s, 1H; C*H*Ph), 6.82–7.01 (m, 5H; Ph-*H*), 7.16–7.57 (m, 5H; Ph-*H*); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=10.4.

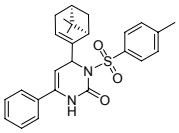


Diethyl N-1,2-diphenylethylidenephosphoramidate (**112**): ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.24 (dt, *J*=0.7 Hz, *J*=7.1 Hz, 6H; CH_3), 3.98–4.04 (m, 4H; CH_2O, 4H), 4.20 (s, 2H; CH_2Ph), 7.16–7.57 (m, 8H; Ph-*H*), 7.93–7.95 (m, 2H; Ph-*H*); ^{31}P NMR (101.25 MHz, $CDCl_3$): δ=2.2.



(4R/S,6R) 6-sec-butyl-4-(4-methoxyphenyl)-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (**114a**)

The general HWE/aza-DA procedure was followed using **101a**, (*S*)-**102d**, **103a** and **105a**. The product was purified by column chromatography to afford **114a** in a 1:1.01 mixture of diastereomers. Yield: 60%; Diastereomeric ratio was determined by HPLC analysis (LiChroCART 250-4 Whelk-O 1, *n*hexane/2-propanol 90:10, 1mL/min); t_r=9.33 min (minor), t_r=10.88 min (major); R=2.19, α =1.26; m.p. 149–153°C [EA]; ^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=0.88 (t, *J*=7.3 Hz, 6H; CH_3CH$_2$), 1.10 (dd, *J*=1.1 Hz, *J*=5.8 Hz, 6H; CH_3CH), 1.41–1.52 (m, 4H; CH_2CH$_3$), 2.02–2.08 (m, 2H; C*H*CH$_3$), 2.35 (s, 6H; CH_3Ph), 3.85 (s, 6H; CH_3O), 4.90 (d, *J*=4.5 Hz, 2H; C*H*C), 5.82–5.90 (m, 2H; C*H*N), 6.86 (d, *J*=8.2 Hz, 4H; *o*-C*H* (PhOMe)), 7.06 (d, *J*=8.1 Hz, 4H; *m*-C*H* (Ts)), 7.24–7.29 (m, 10H; Ph-*H*); ^{13}C NMR: (100.61 MHz, $CDCl_3$): δ=11.5, 11.6, 17.9, 18.0, 21.5, 21.5, 26.8, 26.9, 37.9, 38.1, 55.4, 55.4, 59.1, 59.2, 100.0, 100.1, 114.0 (2C), 114.1 (2C), 128.6 (4C), 128.8 (8C), 133.8, 133.9, 136.4 (2C), 138.2, 138.3, 143.9 (2C), 151.0 (2C), 159.6 (2C); IR: (KBr): $\tilde{\nu}$=3239 (m), 2962 (m), 1681 (s), 1509 (m), 1345 (s), 1167 (s); HRMS (EI): *m/z*=414.1603 $[M]^+$, calc. for $C_{22}H_{26}N_2O_4S$=414.1613.



(1S,4R/S,5R)-6,6-dimethylbicyclo[3.1.1]hept-2-en-2-yl)-6-phenyl-3-tosyl-3,4-dihydropyrimidin-2(1H)-one (**114b**)

The general HWE/aza-DA procedure was followed using **101a**, **102a**, (-)-myrtenal (**103j)** and **105a**. The product was purified by column chromatography to afford **114b** in a 10.8:1 mixture of diastereomers (*de* = 83%). Yield: 80%; Diastereomeric excess was determined by HPLC analysis (LiChroCART 250-4 Whelk-O 1, *n*hexane/2-propanol 95:5, 1mL/min); t_r=13.39 min (minor), t_r=16.12 min (major); R=2.92, α=1.25; m.p.: 76–79°C; $[\alpha]^{20}_D$= +118 (*c*=1.0 in $CHCl_3$); ^{1}H NMR: (400.13 MHz, $CDCl_3$): peaks of the major isomer are reported δ=0.76 (s, 3H; C*H*$_3$ myrtenal), 1.13 (s, 3H; C*H*$_3$ myrtenal), 1.96–2.27 (m, 6H; 2 X C*H*$_2$, 2 X C*H* (myrtenal)), 2.34 (s, 3H; C*H*$_3$Ph), 5.15 (d, *J*=6.2 Hz, 1H; C*H*C), 5.50–5.53 (m, 2H; C*H*N and C*H*CH$_2$), 6.69 (s, 1H; N*H*), 7.17–7.34 (m, 7H; Ph-*H*), 7.89 (d, *J*=8.1 Hz, 2H; Ph-*H*); ^{13}C NMR: (100.61 MHz, $CDCl_3$: δ=21.6, 22.0, 26.5, 31.2, 31.6, 38.6, 40.9, 42.5, 60.1, 99.6, 121.3, 125.6 (2C), 129.4 (2C), 129.5 (2C), 129.7 (2C), 129.9, 133.8, 136.1, 137.0, 144.8, 145.4, 150.9; IR (KBr): $\tilde{\nu}$=3259 (m), 2912 (m), 1689 (s), 1669 (s), 1357 (s), 1171 (s), 1089 (m); HRMS (EI): *m/z*=448.1813 $[M]^+$, calc. for $C_{26}H_{28}N_2O_3S$=448.1821.

α,β-unsaturated ketones (**116a-b** and **118a-b**)

The general HWE/aza-DA procedure was followed, but instead of isocyanate **105**, 5N sulfuric acid (1 mL) was added and the reaction mixture was stirred for 1 hour. The mixture was then extracted with diethyl ether (3×20 mL). The combined organic layer was washed with water (20 mL) and dried over Na_2SO4. ^{1}H NMR analysis of the crude reaction mixture revealed that the α,β-unsaturated ketones **116** or **118** had been formed. The analytic data were in agreement with those reported previously.[36]

3.6 References and notes

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27. (a) PM3 semi-empirical calculations suggest that the ΔEHOMO-LUMO of 1-azadiene **104** (R1=Ph, R2=4-MeOPh) and TsNCO is considerably lower compared to ΔEHOMO-LUMO of the same 1-azadiene with

PhNCO; b) The calculations confirm that for intermediates **B** with intermediates **B** with R3=Ts much more negative charge is localised on N3 compared to intermediates **B** with R3=Ph.

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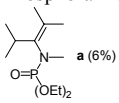
30. Phosphoramidate **a**, with an additional Me-group incorporated was also identified.



a (6%)

31. Next to the phosphoramidates also considerable amounts of the phosphonate **b** were observed.

b (10-20 %)

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4

Synthesis of Thiazines and Dihydropyrimidine-2-thiones

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When instead of isocyanates, isothiocyanates are used in the HWE/aza-DA 4CR, thiazines are formed. These thiazines can be converted into dihydropyrimidinethiones via the Dimroth rearrangement. This rearrangement was investigated with a set of substituted 2-amino-6*H*-1,3-thiazines. The rearrangements were carried out under microwave irradiation conditions in batch or continuous flow format, employing either toluene or 1-methyl-2-pyrrolidone as solvent. Thiazines bearing an ester group at the C5 position rearranged at a considerably higher temperature than derivatives without substituent at this position into the corresponding dihydropyrimidinethiones. This strictly thermal rearrangement was studied in detail using differential scanning calorimetry and density functional theory computational methods. The reaction pathway involves a zwitterionic intermediate.

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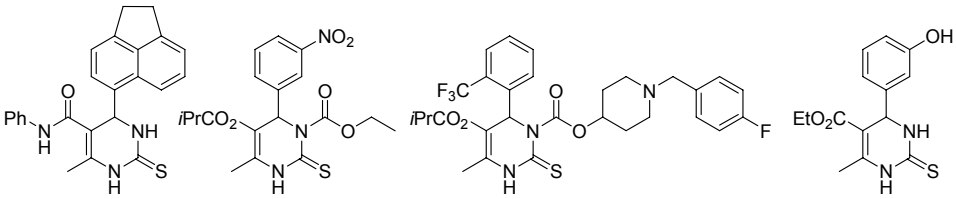
4.1 Introduction

DHPM-2-thiones have an interesting scaffold as is displayed by some biologically active examples (Figure 4.1).[1] DHPM-thione **119** shows colchicine-like properties like destabilising microtubules,[2] while **120** exhibits calcium channel blocking activity, without antihypertensive activity, probably due to rapid metabolism.[3] Further development of the DHPM-2-thiones scaffold led to an orally active long-lasting antihypertensive agent **121**.[4] More recent, monastrol (**122**) has been discovered as cell-permeable molecule that blocks mitosis by specifically inhibiting the motor activity of mitotic kinesin, Eg5, which mediates centrosome separation and formation of the bipolar mitotic spindle.[5]

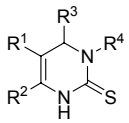


119 **120** **121** **122**

Figure 4.1 Biologically active DHPM-thiones

DHPM-2-thiones like **119-121** can be synthesised via the Biginelli 3CR and as with DHPMs, additional manipulations are necessary to functionalise N3.[4-6] A large range of variations of the original Biginelli 3CR using several catalysts, solid phase chemistry, neat conditions or microwave irradiation are known to overcome the relatively low yields of the original 3CR.[7] Obviously, a more direct synthetic route to generate DHPM-2-thiones **123** with the desired substitution pattern would greatly facilitate further pharmacological studies, *e.g*. SAR studies.

In the former chapter the synthesis of DHPMs via a HWE/aza-DA 4CR has been described. The related dihydropyrimidine-2-thiones (DHPM-2-thione; **123**) should become available by the application of isothiocyanates instead of isocyanates in the 4CR.



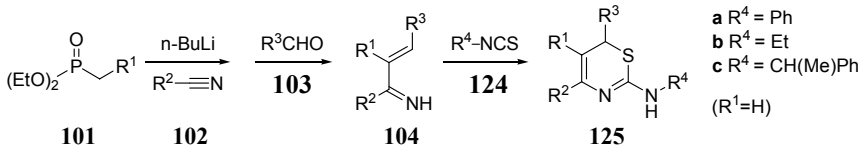
123

Figure 4.2 DHPM-2-thione scaffold

4.2 Results and discussion

4.2.1 Synthetic aspects

Thus, we decided to explore the reactivity of isothiocyanates (**124**) in the HWE/aza-DA 4CR. The reaction of phosphonates, nitriles, aldehydes and isothiocyanates did not result in the formation of DHPM-2-thiones **123**. Instead thiazines **125** were formed (Scheme 4.1 and Table 4.1).



Scheme 4.1 Synthesis of thiazines

The procedure proved quite general and a number of differently substituted thiazines **125** could be synthesised in reasonable to good yields by combining the appropriate inputs (Table 4.1). By simply changing the nature of the fourth component in our MCR from isocyanate to isothiocyanate the course of the final cyclocondensation is altered. Interestingly, not only electron withdrawing but also alkyl R^4-substituents are tolerated in the formation of thiazines **125**, which is in contrast to the 4CR towards DHPMs.

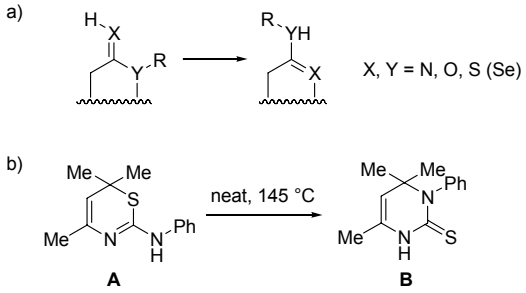
Table 4.1. HWE/aza-DA 4CR of phosphonate **101a** with nitriles **102**, aldehydes **103** and isothiocyanates **124**.

Entry	Nitrile	R^2	Aldehyde	R^3	Isothio-cyanate	R^4	Thiazine	Yield (%)
1	**102a**	Ph	**103c**	Ph	**128a**	Ph	**125a**	74
2	**102a**	Ph	**103a**	4-MeOPh	**128a**	Ph	**125a**	58
3	**102b**	*i*Pr	**103c**	Ph	**128a**	Ph	**125c**	61
4	**102a**	Ph	**103a**	4-MeOPh	**128b**	Et	**125d**	56
5	**102a**	Ph	**103a**	4-MeOPh	**128c**	CH(Me)Ph	**125e**	64

Thiazines are scarcely described in literature,[8,9] and not much is known on their biological activities.[10,11] Our synthetic approach facilitates access to **125** and should promote further studies toward their biological relevance. In general thiazines **125** are rather stable compounds and they can be easily purified by CC, however, in the case of **125b** (entry 2; Table 4.1) a rearrangement to the corresponding DHPM-2-thione was observed upon standing at room temperature for a couple of hours in $CDCl_3$.

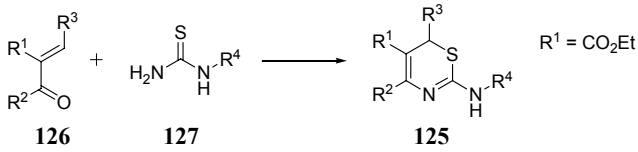
These results in relation to the earlier work by Kappe *et al.*[9] prompted us to explore the rearrangement of thiazines to DHPM-2-thiones in more detail and to investigate its scope as preparative route to *N*3-substituted DHPM-2-thiones.[12,13] In the remainder of this chapter we present our computational, mechanistic and experimental results on this so-called Dimroth rearrangement of two series of 2-amino-1,3-thiazines.

The Dimroth rearrangement is an isomerisation process whereby exo- and endocyclic heteroatoms are translocated on a heterocyclic ring. In general, it involves the translocation of exo- and endocyclic heteroatoms (most commonly nitrogen) on a single heterocyclic ring, and has been referred to as an exoannular rearrangement (Scheme 4.2a)[14,15] The isomerisation process is usually promoted by the presence of base or acid in solution, but may also be performed under strictly thermal or photochemical conditions. Many factors such as the nature and number of heteroatoms in the ring, or the presence of electron-withdrawing substituents on the heterocycle influence the Dimroth rearrangement.[14,15] Although early reports of the Dimroth rearrangement date back to as early as 1888,[16,17] it was only recognised as a general phenomenon in heterocyclic chemistry in the 1950's and has since been reviewed several times.[14] Although the Dimroth rearrangement is most commonly associated with all-nitrogen heterocycles such as aminoazoles (imidazoles, triazoles) and aminoazines (pyridines, pyrimidines),[14,15] there are a few examples of Dimroth rearrangements known involving the transformation of amino-1,3-thiazines to pyrimidinethiones.[18-20] A particularly interesting example involves the thermal rearrangement of 2-amino-1,3-thiazine **A** to 3,4-dihydropyrimidine-2-thione **B** reported by Zigeuner and co-workers in 1975 (Scheme 4.2b).[19,20] Simply heating above its melting point at 145°C resulted in Dimroth rearrangement of thiazine **A** to the corresponding pyrimidine **B** in moderate yields.



Scheme 4.2 a) Translocation of exo- and endocyclic heteroatoms in a heterocyclic ring. b) Dimroth rearrangement of 2-amino-1,3-6*H*-thiazine **A** to 3,4-dihydropyrimidine-2-thione **B**.

To investigate the Dimroth rearrangement of thiazines an additional series of thiazines **125g–l** was synthesised according to the method outlined in Scheme 4.3. These thiazines having an ester group at *C*5 of the thiazine nucleus could be rapidly prepared by condensation of enones and thioureas in acidic medium.[9]



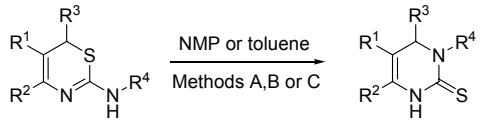
126 **127** **125**

Scheme 4.3 Second pathway to 2-amino-1,3-6*H*-thiazines

In this second protocol also sometimes the formation of the corresponding rearranged DHPMs as by-product was observed upon careful investigation of the crude reaction mixtures. We therefore investigated this isomerisation in more detail employing several isolated and purified thiazine derivatives **125** as model compounds. Since some of the Dimroth rearrangements performed in organic solvents required quite high temperatures (see below) we employed controlled microwave heating under sealed vessel conditions to rapidly attain those temperatures.[21] It quickly became evident that thiazines **125a–f**, lacking an ester functionality at C5, required considerably lower temperatures for rearrangement than thiazines **125g–l** that have the electron-withdrawing ester group. This was confirmed by Differential Scanning Calorimetry (DSC) experiments and also substantiated by computational methods (see below).

For preparative purposes three different rearrangement protocols were developed that lead to DHPMs **123a–l** in 55-95 % isolated yields (Table 4.2). After some experimentation with a variety of different solvent systems and microwave irradiation conditions it became clear that these monomolecular rearrangements are best conducted in either toluene or 1-methyl-2-pyrrolidone (NMP) at a concentration level of ca 100 mmol/L. As indicated, thiazines **125a–f** without an ester group at C5 rearrange at considerably lower temperatures (120°C) than thiazines **125g–l** in a variety of different solvents. For consistency, all experiments were repeated in toluene, which allowed clean rearrangement of **125a–f** at 120°C within 60 minutes. For *N*-substituted thiazines **125g–j** (R^4 = alkyl or aryl) NMP at 200°C proved to yield the corresponding thiones **123g–j** more efficiently, while thiazines **125k**,**l** (R^4 = H) were best transformed into DHPMs in a non-polar solvent such as toluene at 210°C.

Table 4.2 Microwave-assisted rearrangement of 2-amino-1,3-6*H*-thiazines **125** to 3,4-dihydropyrimidine-2-thiones **123**.[a]



125a–l **123a–l**

Thiazine	R^1	R^2	R^3	R^4	Method[b]	Yield (%)[c]
125a	H	Ph	Ph	Ph	A	74
125b	H	Ph	4-MeOPh	Ph	A	67
125c	H	*i*Pr	Ph	Ph	A	59
125d	H	Ph	4-MeOPh	Et	A	70
125e	H	Ph	4-MeOPh	CH(Me)Ph	A	55
125f	H	Ph	Ph	Et	A	72
125g	CO_2Et	Ph	Ph	Et	B	87
125h	CO_2Et	Me	Ph	Me	B	93
125i	CO_2Et	Me	4-MePh	Me	B	95
125j	CO_2Et	Me	Ph	Ph	B	61
125k	CO_2Et	Me	Ph	H	C	67
125l	CO_2Et	Ph	Ph	H	C	63

a) Single mode microwave heating (MW) under sealed vessel conditions, 0.08 – 0.20 mmol scale; b) Method A: toluene, MW, 120°C, 45-60 min; Method B: NMP, 200°C, 35 min; Method C: toluene, MW, 210 °C, 40 min; c) Isolated yield of pure compound. See Experimental Section for more details.

4.2.2 Kinetic and Differential Scanning Calorimetry investigations

As mentioned above, it became apparent early on in our investigations that thiazines **125** without an ester group at *C*5 (**125a**–**f**, R^1 = H) rearrange at distinctly lower temperature than the C5-substituted analogues (**125g–l**, $R^1 = CO_2Et$). In order to determine and compare the exact rearrangement temperatures, we compared two model thiazines that had an identical substitution pattern for R^2 (Ph), R^3 (Ph) and R^4 (Et), but differed in the R^1 substituent: **125f** (R^1 = H) and **125g** ($R^1 = CO_2Et$). Then both thiazines **125f** and **125g** were subjected to the microwave-assisted rearrangement conditions in NMP for a fixed time of 30 min at variable temperatures (Table 4.3). By HPLC-monitoring of the formation of DHPMs **123f** and **123g,** respectively, it was found that thiazine **125f** completely rearranges at 120°C within the 30 min timeframe of the experiment. On the other hand, thiazine **125g** bearing the ester group at C5 required a significantly higher reaction temperature (ca 200°C) for full conversion to the corresponding DHPM-2-thione **123g** (Table 4.3).

Table 4.3 Comparison of Rearrangement Temperatures for Thiazine **125f** (R^1=H) and **125g** (R^1=CO_2Et).[a]

Thiazine	Temp (°C)	Conversion (%)[b]
125f	80	0
	100	16
	120	100
125g	140	2
	160	41
	180	76
	200	100

a) Conditions: NMP, MW, 30 min, 80-200 °C, 100 mmol/L thiazine; b) HPLC conversion (254 nm, peak area percent).

To further support these data, DSC measurements on selected thiazines were performed. The DSC technique, widely used in material science and process research, has recently also emerged as useful tool for synthetic organic chemists.[22] It provides information on the expected temperature, at which a reaction will take place, especially in the case of monomolecular transformations such as ring closure or rearrangement reactions. Of particular interest to us was the direct comparison of the DSC plots of thiazines **125f** and **125g** shown in Figure 4.3.

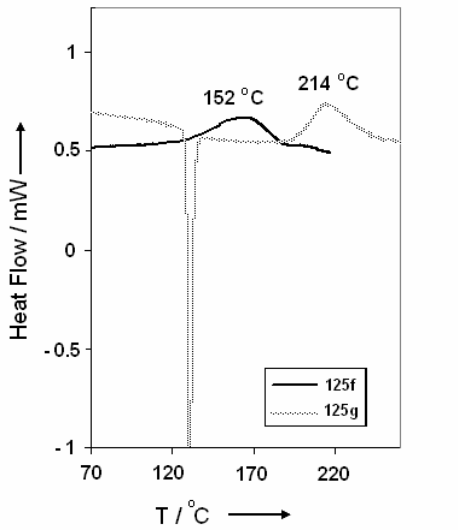
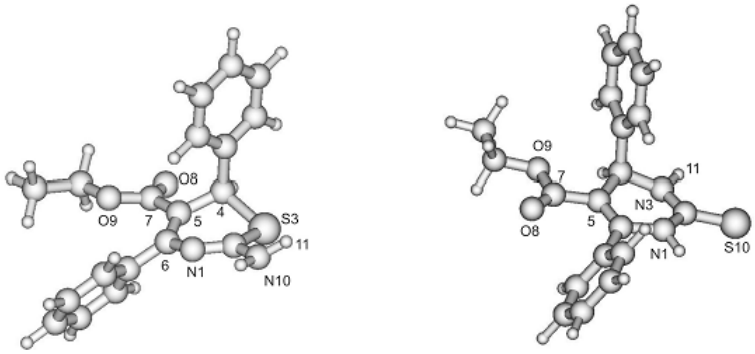


Figure 4.3 Comparison of differential scanning calorimetry (DSC) plots of thiazines **125f** and **125g**. The onset point for the solid-state rearrangement of thiazines **125f** and **125g** are 142 and 197 °C, respectively. The measured reaction enthalpy (ΔH) was 16 J/mmol for **125f** → **123f** and 19 J/mmol for **125g** → **123g**

The DSC curve of the solid thiazine compound **125g** exhibited a sharp endothermic peak at 130°C (peak maximum) marking the melting point of the thiazine, followed by an exothermic peak, which had a maximum at 214°C. This latter peak marked the rearrangement of the thiazine **125g** to the corresponding DHPM **123g** (onset point of the rearrangement at 197°C) and corresponded well with the data obtained in NMP solution (Table 4.3). In contrast, the DSC curve of thiazine **125f** (oil) showed no melting point and displayed an exothermic peak marking rearrangement to **123f** at 152°C (peak maximum, onset point at 142°C), again nicely matching the data obtained in NMP solution (Table 4.3). Similar DSC diagrams were obtained from thiazines **125a, 125h, 125j** and **125k** further corroborating the differences in rearrangement temperatures between thiazines **125** with and without ester substitution at *C*5.

4.2.3 Mechanism of Rearrangement - Computational Studies

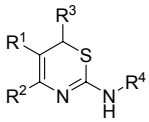
In order to rationalise the difference in rearrangement temperatures and to gain further insight into the mechanism of this particular type of Dimroth rearrangement a computational investigation was carried out on the 2-amino-1,3-6*H*-thiazine to 3,4-dihydropyrimidine-2-thione rearrangement. After initial geometry optimisations of **125f**, **125g** and **125l** and rearranged products **123f**, **123g**, and **123l** it became clear that both the thiazines **125** as well as the DHPM-2-thiones **123** can exist in various conformations. The different conformers originate from different orientations of the ester and/or amino/imino groups, which are characterised by the respective dihedral angles τ_1=τ(C6–C5–C7–O8) and τ_2=τ(N1–C2–N10–C11). Representative structures of **125l** and **123l** and atom numbering are displayed in Figure 4.4 (Note that S3 and N10 of the reactants correspond to S10 and N3 in the products). The computed structures agree well with X-ray data on related thiazines[8] and DHPMs.[23]



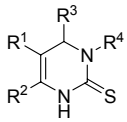
Thiazine **125l** Dihydropyrimidine **123l**

Figure 4.4 Selected optimised geometries (B3LYP/6-31G(d)) of thiazine **125l** and DHPM **123l**

Next to the different conformations the amino-imino (for **125**) and thion-thiol (for **123**) tautomerism was taken into account. Clearly, for **125f**, **125g** and **125l** the amino tautomers are more stable than the corresponding imino forms with an exocyclic C2=N10 double bond. In contrast, for **123f**, **123g**, and **123l** the thione tautomers with an exocyclic C2=S10 double bond are more favourable than thiols. Furthermore, the thiol tautomer of **123l** that bears a proton at *N*3 is favored. Also, calculations show that in some cases the *s-trans* ester conformer is more stable (**125l** and **123l**) whereas in other derivatives (e.g., those with R^2 = *i*-propyl) the *s-cis* rotamer is favoured. Indeed for similar systems,[24] both the *s-cis* and the *s-trans* rotamer are reported. From the computational data it is evident that rearrangement products **123** are considerably more stable than the respective thiazines **125**. Thus the described Dimroth-type rearrangement is exothermic and exergonic, as also confirmed by the DSC measurements. However, product stability arguments alone cannot explain the differences in rearrangement temperatures observed for the two series of compounds. To clarify the influence of substituents, especially the ester group at *C*5 of the heterocyclic ring on the rearrangement process, detailed calculations (B3LYP/6-31G(d)) on model systems (aminothiazines **128a–d**, their imino tautomers **128a–1, 128b–1, 128c–1, 128d–1**, and the corresponding rearranged pyrimidine-2-thiones **129a–129d**) were performed (Figure 4.5).



128



129

128/129	R^1	R^2	R^3	R^4
a	H	H	H	H
b	CO_2Et	H	H	H
c	H	H	Ph	H
d	CO_2Et	H	Ph	H

Figure 4.5 Structures of model compounds **128a – d** and **129a – d**.

In the following, a purely thermal reaction path for the transformation 2-aminothiazine → pyrimidine-2-thione, i.e. without the involvement of any nucleophile or electrophile, is described. Relative energies of reactants, transition states, intermediates, and products for the transformations **128a–128d → 129a–129d** have been calculated (B3LYP/6-31G(d)) and the reaction paths for the rearrangements **128c→129c** and **128d→129d** are shown in Figure 4.6. From the computational results it was not possible to locate a mechanistic pathway for direct rearrangement of **128** to **129**. Instead, rearrangement seems to proceed *via* the corresponding imino tautomers of **128**. Thus, after initial tautomerisation of **128a–128d → 128a-1**–**128d-1**, rearrangement commences by rotation of the thioimide group S3–C2=N10 around the N1–C2 bond to give, via **TS1a**–**TS1d**, the intermediates **Int-a**–**Int-d**. Subsequent ring closure of these intermediates via **TS2a–TS2d** leads to the rearranged products, **129a–129d**. Activation energies are in the range 35–40 kcal mol^{-1} and thus, can easily overcome the energy requirements for the necessary tautomerisation prior to rearrangement. Careful analysis of the computational data reveals that an ethoxycarbonyl group at *C*5 raises the activation energies for rearrangement by ca. 3 kcal mol^{-1} compared to those with a H-atom at *C*5. The ethoxycarbonyl group at *C*5 raises both activation energies, especially that of **TS1**. Thus, for the rearrangement **128a→129a** the second step is rate determining, for **128b→129b** both steps have almost equal activation energies, and for reactions **128c→129c** and **128d→129d** ring opening of the imino tautomers of the thiazines to the respective intermediates **Int-c** and **Int-d** is rate determining.

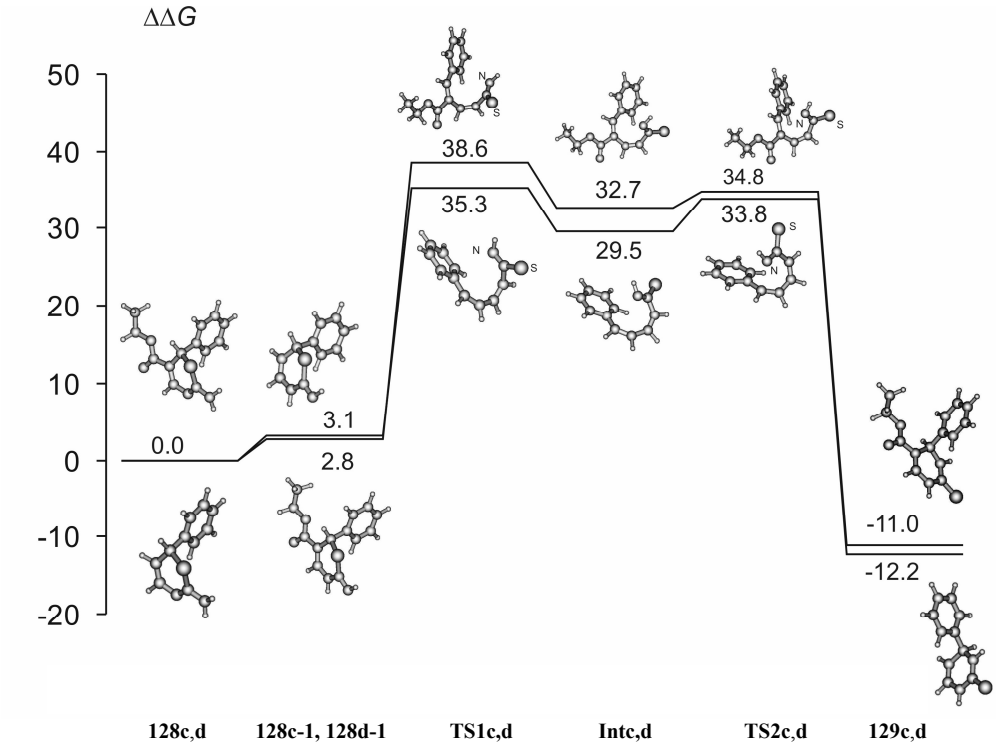


Figure 4.6 Calculated reaction paths for the thermal aminothiazine – pyrimidinethione rearrangements **128c→129c** and **128d→129d** (energies in kcal mol^{-1})**.**

Most importantly, the sum of the activation energies for the rearrangement of derivatives with an ester group at C5 is higher by ca. 3 kcal mol^{-1} compared to rearrangement of derivatives lacking this substituent. Isodesmic/homodesmotic reactions (1) and (2) and analogous ones for the respective transition states **TS1**, **TS2**, intermediates and products were used to estimate the effect of introducing the ethoxycarbonyl group. From the computational data it may be concluded that the increased activation energy for rearrangement of 5-ethoxycarbonylthiazines as compared to their C5–unsubstituted derivatives results both from a stabilisation of the reactant as well as a destabilisation of the transition states by CO_2Et.

128a-1 + ethyl acrylate (*s-cis* and *s-trans*) → **128b-1** + ethylene (1)

128c-1 + ethyl acrylate (*s-cis* and *s-trans*) → **128d-1** + ethylene (2)

The question now remains whether the influence of the ester group on reactant and transition state energies can account for the different rearrangement temperatures found for the two series of compounds. For this purpose it is useful to consider the geometric and electronic features of the transition states involved in this purely thermal rearrangement. The electronic structures have been analysed with the NBO method.[25] Ring opening of the 2-iminothiazine by rotation of the thioimide moiety results in a substantial structural and electronic change: *r*(N1–C2) and *r*(C5–C6) are significantly longer in **TS1**, **Int** and **TS2** than in the starting 2-iminothiazine and the rearranged pyrimidine-2-thione, whereas *r*(N1–C6) and *r*(C4–C5) are shorter. In line with these geometrical changes, the NBO analysis indicates the presence of double bonds between N1=C6 and C4=C5, and single bonds between N1–C2 and C5–C6. Figure 4.7 gives a pictorial representation of the bonding pattern in **TS1.**

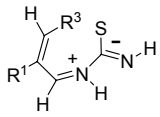


Figure 4.7 Charge distribution (NPA charges) and bonding pattern (NBO analysis) for transition states **TS1a–d** (an analogous representation of the charge distribution is also indicated for the intermediates **Int** and the second transition states, **TS2**).

As a consequence, both transition states **TS1** and **TS2** as well as the intermediate **Int**, have a pronounced zwitterionic character. For instance, in the C5-unsubstituted derivatives (R^3 = H), the negative charge on the S3-C3=N10H fragment is ca. –0.40 (Mulliken) and –0.46 (NPA); in contrast, in the reactants this moiety bears a small positive charge (ca. + 0.1). Conversely, the R^1–C4–C5–C6–N1 fragment is positively charged in **TS1**, **Int**, and **TS2**, and negatively charged in the reactants. This means that introduction of an electron–withdrawing substituent will stabilise/destabilise a negatively/positively charged moiety. Hence, the C5-located ester group (CO_2Et) lowers the energy of the reactant and increases that of the transition states, leading to higher rearrangement temperatures for the ester substituted derivatives.

4.3 Conclusions

A range of differently substituted 2-amino-6*H*-1,3-thiazines were efficiently transformed into 3,4-dihydropyrimidine-2-thiones via a Dimroth-type rearrangement. Isolated yields of DHPM-thiones **123** were reasonable to good. In principle, arrays of privileged scaffolds are now accessible in only two steps, taking into account that the starting thiazines **125** can be prepared by highly versatile multicomponent reactions. DSC measurements can be used to

determine the optimal temperature at which Dimroth rearrangement takes place. This temperature seems to depend mainly on the C5 substituent. Thiazines with an ester at C5 rearrange at significantly higher temperatures then thiazines with a proton at C5. The described rearrangement is a strictly thermal process that most likely proceeds via a zwitterionic intermediate as was corroborated by DFT calculations.

4.4 Acknowledgements

We thank Dr. Marek Smoluch for measuring the HRMS samples. We thank Prof. W. Stadlbauer for assistance in performing and interpreting the DSC experiments. We also thank Biotage AB (Uppsala, Sweden) for the provision of an Initiator 8 microwave reactor and Michael Collins Jr. (CEM Corporation) for technical advice in performing continuous flow microwave experiments

4.5 Experimental Section

4.5.1 General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 (250.13 and 62.90, respectively) or a Bruker MSL 400 (400.13 and 100.61 MHz respectively) spectrometer; chemical shifts (δ) are given in ppm, internally referenced to residual solvent resonances (^{1}H: δ 7.29 ppm, ^{13}C: δ 77.0 ppm). Column chromatography was performed on Baker 7024-02 (40 μ, 60 Å) or Merck 60H silica gel with petroleum ether (PE, boiling range 40-60 °C), ethyl acetate (EA) and cyclohexane (*c*-hexane) as eluents. Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC plates were visualised under UV light or by treatment with an anisaldehyde solution. IR spectra (KBr or NaCl) were recorded on a Mattson 6030 Galaxy spectrophotometer and are reported in cm^{-1}. High-resolution mass spectra were measured at 70 eV with a Finnigan MAT900 spectrometer. Low-resolution mass spectra were obtained in the atmospheric pressure chemical ionisation (positive or negative APCI mode). Analytical HPLC analysis was carried out on a LiChrospher 100 C18 reversed-phase analytical column (119 × 3 mm, 5μm particle size) at 25 °C, using mobile phase A (water/MeCN 9:1 (v/v) + 0.1% TFA) and phase B (MeCN + 0.1% TFA), with linear gradient from 30% B to 100% B in 8 min and 2 min with 100% phase B. Melting points were measured on a Stuart Scientific SMP3 melting point apparatus and are uncorrected. Calorimetric (DSC) data were obtained on a Rheometric Scientific DSC-Plus instrument (software - Orchestrator v.6.5.8) using thiazines **125** in 1.5 – 3 mg amounts in closed crucibles. The DSC plots were taken in the range of 30 – 350 °C at heating rate 10 °C/min. Tetrahydrofuran (THF) was dried and distilled from sodium benzophenone prior to use. PE was distilled prior to use. Benzonitrile (**102a**), isobutyronitrile (**102d**) and benzaldehyde (**103c**) were dried, distilled, and stored under a dry nitrogen atmosphere. Other commercially available chemicals were used as purchased.

4.5.2 Microwave Experiments

Microwave-assisted reactions were performed in either an Initiator 8 (Biotage AB) or Discover (CEM Corporation) single-mode microwave instrument at 2450-MHz controlled irradiation using standard sealed microwave glass vials. Reaction temperatures were monitored by an IR sensor on the outside wall of the reaction vials. Reaction times refer to hold times at the selected set temperature, not to total irradiation times.

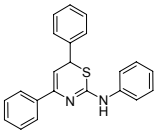
4.5.3 Calculations

All calculations were done with the aid of the Gaussian G03 program suite.[26] Geometries were completely optimised by Becke's three-parameter hybrid HF – density functional theory[27] method in combination with the Lee-Yang-Parr correlation functional[28] and the 6-31G(d) basis set. All structures were characterised as true minima or transition states by vibrational frequency calculations. For transition states, in addition, intrinsic reaction coordinate calculations were performed. The electronic structures were analysed with the NBO method.[25,29]

4.5.4 Synthetic Procedures and Physical Data

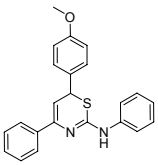
General procedure for the synthesis of thiazines 125a-f

*n*BuLi (1.6 M in hexane; 1.2 equiv) was added dropwise to a stirred solution of phosphonate **101a** (0.2 M in dry THF) at –78°C. The resulting solution was stirred for 1.5 h and then nitrile **102** (1.1 equiv) was added. The reaction mixture was allowed to warm to –5°C over 1.5 h and then aldehyde **103** (1.1 equiv) was added. The resulting mixture was stirred for a further 0.5 h at –5°C and thereafter for 1.5 h at room temperature. Isothiocyanate **128** (1.1 equiv) was then added dropwise over 10 min and the resulting solution was stirred overnight. The solvent was removed under reduced pressure and the crude product was isolated by column chromatography (PE/EA 10/1 → 3/1).



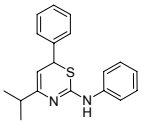
***N*-4,6-triphenyl-6*H*-1,3-thiazin-2-amine (125a)**

Yield: 74%; m.p. 128–129 °C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=5.03 (d, *J*=5.6 Hz, 1H; C*H*C), 5.76 (d, *J*=5.6 Hz, 1H; C*H*S), 7.11 (t, *J*=7.3 Hz, 1H; Ph-*H*), 7.29–7.57 (m, 12H; Ph-*H*), 7.79 (d, *J*=6.9 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=44.5, 101.1, 120.9 (2C), 123.7, 125.9 (2C), 127.9 (2C), 128.1, 128.4, 128.5 (2C), 128.9 (4C), 138.2, 140.7, 141.5, 145.6, 150.0; IR (KBr): $\tilde{\nu}$=1574 (s), 1493 (w), 1292 (w), 1205 (w), 756 (m), 692 (m); HRMS (EI): *m/z*=342.1187 $[M]^+$, calc. for $C_{22}H_{18}N_2S$=342.1191.



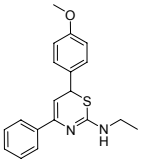
6-(4-methoxyphenyl)-*N*,4-diphenyl-6*H*-1,3-thiazin-2-amine (125b)

The product was isolated as a yellow-brown oil. Yield: 58%; ^{1}H NMR (400.13 MHz, DMSO): δ=3.70 (s, 3H; CH_3), 5.11 (d, *J*=6.5 Hz, 1H; C*H*C), 5.91 (d, *J*=6.5 Hz, 1H; C*H*S), 6.88 (d, *J*=8.6 Hz, 2H; *m*-C*H* (PhOMe)), 6.96-6.99 (m, 1H; *p*-C*H* (NHPh)), 7.25–7.40 (m, 7H; Ph-*H*), 7.78–7.81 (m, 4H; *o*-C*H* (NHPh and Ph)), 9.32 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, DMSO): δ=42.0, 55.1, 101.1, 114.0 (2C), 119.5 (2C), 122.3, 125.3 (2C), 127.7, 128.3 (2C), 128.5 (2C), 128.6 (2C), 133.6, 139.1, 140.5, 146.2, 148.4, 158.7; IR (NaCl): $\tilde{\nu}$=2961 (w), 1577 (s), 1509 (s), 1253 (s), 1030 (s). HRMS (EI): *m/z*=372.1230 $[M]^+$, calc. for $C_{23}H_{20}N_2OS$=372.1296



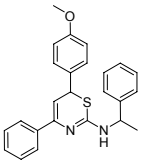
4-isopropyl-*N*,6-diphenyl-6*H*-1,3-thiazin-2-amine (125c)

Yield: 61 %; m.p. 124–128 °C [EA]; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.20 (d, *J*=6.9 Hz, 6H; C*H*$_3$), 2.40–2.61 (m, 1H; C*H*(CH$_3$)$_2$), 4.77 (d, *J*=4.9 Hz, 1H; C*H*C), 4.99 (d, *J*=4.9 Hz, 1H; C*H*S), 7.01–7.29 (m, 10H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=21.5, 21.6, 34.3, 44.3, 97.7, 121.6, 123.8, 128.2 (2C), 128.3 (2C), 129.1 (2C), 129.2 (2C), 139.3, 141.2, 149.9, 150.3; IR (KBr): $\tilde{\nu}$=3201 (w), 2962 (w), 1603 (s), 1581 (s), 1489 (m), 1309 (m), 1194 (m); HRMS (EI): *m/z*=308.1354 [M]$^+$, calc. for $C_{19}H_{20}N_2S$=308.1347.



***N*-ethyl-6-(4-methoxyphenyl)-4-phenyl-6*H*-1,3-thiazin-2-amine (125d)**

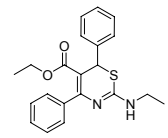
The product was isolated as yellow oil. Yield: 56%; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.23 (t, *J*=7.3 Hz, 3H; C*H*$_3$CH$_2$), 3.57–3.60 (m, 2H; C*H*$_2$), 3.78 (s, 3H; C*H*$_3$O), 4.88 (d, *J*=5.8 Hz, 1H; C*H*S), 5.71 (d, *J*=5.8 Hz, 1H; C*H*CHS), 6.84 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.25–7.36 (m, 5H; Ph-*H*), 7.80 (d, *J*=7.4 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=14.9, 37.3, 43.9, 55.3, 99.9, 114.2 (2C), 125.6 (2C), 127.8, 128.1 (2C), 128.9 (2C), 133.5, 139.4, 147.4, 152.4, 159.2; IR (NaCl): $\tilde{\nu}$=3400 (w), 2969 (w), 1567 (s), 1510 (s), 1249 (s); HRMS (EI): *m/z*=324.1310 [M]$^+$, calc. for $C_{19}H_{20}N_2OS$=324.1296.



6-(4-methoxyphenyl)-4-phenyl-*N*-(1-phenylethyl)-6*H*-1,3-thiazin-2-amine (125e)

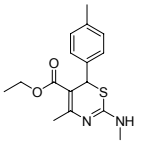
The product was isolated as yellow oil as a 1:1 mixture of diastereomers. Yield: 64%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.54 (d, *J*= 7.2 Hz, 3H; CHC*H*$_3$Ph), 1.56 (d, *J*=7.5 Hz, 3H; CHC*H*$_3$Ph), 3.79 (s, 3H; OC*H*$_3$), 3.80 (s, 3H; OC*H*$_3$), 4.85 (d, *J*=6.0 Hz, 1H; C*H*S), 4.88 (d, *J*=5.6 Hz, 1H; C*H*S), 5.38–5.41 (m, 2 × 1H; C*H*CH$_3$Ph), 5.68 (d, *J*=5.6 Hz, 1H; C*H*CHS), 5.71 (d, *J*=6.0 Hz, 1H; C*H*CHS), 6.81–7.60 (m, 28H; Ph-*H*); ^{13}C NMR (400.13 MHz, $CDCl_3$, 25 °C): δ=22.7, 22.8, 43.8, 44.1, 51.9 (2C), 55.3, 55.4, 99.8, 100.3, 114.1 (2C), 114.2 (2C), 125.7 (4C), 126.0 (2C), 126.1 (2C), 127.0, 127.1, 127.8 (2C), 128.0 (4C), 128.5 (2C), 128.6 (2C), 128.9 (4C), 133.3, 133.4, 139.1 (2C), 144.0, 144.1, 150.3, 150.7, 159.2 (2C), 188.7 (2C); IR (NaCl): $\tilde{\nu}$=3399 (w), 2929 (w), 1567 (s), 1509 (s), 1250 (s); HRMS (EI): *m/z*=400.1612 [M]$^+$, calc. for $C_{25}H_{24}N_2OS$=400.1609.

Thiazines **125g**, **125i** and **125l** have been prepared according to ref. 9c.



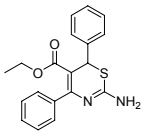
Ethyl 2-(ethylamino)-4,6-diphenyl-6H-1,3-thiazine-5-carboxylate (125g).

Yield: 71%; m.p. 135–137°C [*i*-propanol]; ^{1}H NMR (360 MHz, $CDCl_3$): δ=0.85 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 1.10 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 3.46 (br s, 2H; C*H*$_2$CH$_3$), 3.83–3.96 (m, 2H; C*H*$_2$CH$_3$), 4.81 (br s, 1H; N*H*), 5.41 (s, 1H, C*H*S), 7.22–7.40 (m, 8H; Ph-*H*), 7.47–7.51 (m, 2H; Ph-*H*); ^{13}C NMR (90 MHz, $CDCl_3$): δ=13.5, 14.8, 37.8, 43.7, 60.2, 126.8 (2C), 127.5 (2C), 128.0, 128.5, 128.6 (4C), 141.6, 141.9, 158.4, 168.0;[30] MS (pos APCI): *m/z*=368 (11, M+2), 367 (27, M+1), 366 (100, M), 334 (33, M–32), 279 (38, M–87). Anal. Calcd for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64. Found: C, 68.96; H, 5.95; N, 7.56.



Ethyl 4-methyl-2-(methylamino)-6-p-tolyl-6*H*-1,3-thiazine-5-carboxylate (125i).

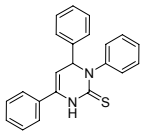
Yield: 72%; m.p. 119–120°C [EtOH]; ^{1}H NMR (360 MHz, $CDCl_3$): δ=1.23 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.30 (s, 3H; C*H*$_3$), 2.55 (s, 3H; C*H*$_3$), 2.98 (s, 3H, C*H*$_3$), 4.07–4.20 (m, 2H, C*H*$_2$CH$_3$), 4.76 (br s, 1H; N*H*), 5.28 (s, 1H; C*H*S), 7.03–7.09 (m, 4H; Ph-*H*); ^{13}C NMR (90 MHz, $CDCl_3$): δ=14.3, 21.1, 24.3, 42.5, 60.1, 126.7 (2C), 129.2 (2C), 137.1, 139.2, 167.2;[30] MS (pos APCI) *m/z* 304 (100, M), 231 (10, M–73).



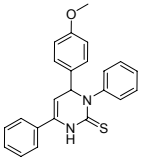
Ethyl 2-amino-4,6-diphenyl-6*H*-1,3-thiazine-5-carboxylate (125l).
Yield: 66%; m.p. 253-255°C (dec) [*i*-propanol]; ^{1}H NMR (360 MHz, DMSO-d_6): δ=0.74 (t, *J*=7.0 Hz, 3H; C*H*$_3$), 3.56 (br s, 2H; N*H*$_2$), 3.83 (q, *J*=7.0 Hz, 2H; C*H*$_2$), 5.75 (s, 1H; C*H*S), 7.33–7.55 (m, 10H; Ph-*H*); ^{13}C NMR (90 MHz, DMSO-d_6): δ=13.7, 41.8, 61.1, 127.2 (3C), 128.8 (3C), 129.4 (2C), 129.6 (2C), 130.6, 139.8, 165.7;[30] MS (pos APCI) *m/z* 338 (100, M), 304 (27, M–34), 279 (19, M–59).

General Procedures for the Dimroth Rearrangement of 2-Amino-1,3-thiazines 125a–l into Dihydropyrimidine-2-thiones 123a–l.

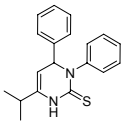
Method A (for compounds **125a–f**): In a 5 mL pyrex microwave vial a sample of the corresponding thiazine (0.64 mmol) was dissolved in 1.5 mL of dry toluene. The vial was sealed and subjected to MW irradiation at 120 °C for 45–60 min. After the reaction was completed the solvent was removed under reduced pressure and the residue subjected to column chromatography (cyclohexane/EtOAc (19:1)) to obtain products **123a–f** as pale yellow oils.



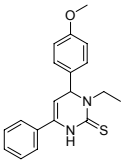
3,4,6-Triphenyl-3,4-dihydropyrimidine-2(1*H*)-thione (123a).
The product was isolated as a pale yellow oil. Yield: 74%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=5.34 (d, *J*=4.9 Hz, 1H; C*H*C), 5.44 (dd, *J*=4.9 Hz, *J*=2.0 Hz, 1H; C*H*N), 7.02–7.05 (m, 2H; Ph-*H*), 7.15–7.52 (m, 13H; Ph-*H*), 7.92 (s, 1H; N*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=66.1, 101.0, 125.0 (2C), 127.5 (2C), 127.8, 128.2, 128.5 (2C), 128.8 (2C), 129.0 (4C), 129.5, 133.0, 133.6, 141.1, 143.9, 176.7; IR (NaCl): $\tilde{\nu}$=1698 (s), 1491 (s), 1455 (s), 1230 (s); HRMS (EI): *m/z* = 342.1191 (M^+), calc. for $C_{22}H_{18}N_2S$ = 342.1191.



4-(4-Methoxyphenyl)-3,6-diphenyl-3,4-dihydropyrimidine-2(1*H*)-thione (123b).
The product was isolated as a pale yellow oil. Yield: 67% yield; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=3.81 (s, 3H; OC*H*$_3$), 5.28 (d, *J*=4.9 Hz, 1H; C*H*C), 5.40 (dd, *J*=4.9 Hz, *J*=2.2 Hz, 1H; C*H*N), 6.82 (d, *J*=8.7 Hz, 2H; Ph-*H*), 6.99–7.01 (m, 2H; Ph-*H*), 7.13 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.26–7.36 (m, 3H; Ph-*H*), 7.41–7.45 (m, 5H; Ph-*H*), 7.48–7.51 (m, 5H; Ph-*H*), 7.92 (s, 1H; N*H*); ^{13}C-NMR (100.61 MHz, $CDCl_3$): δ=55.7, 66.1, 101.8, 114.6 (2C), 125.6 (2C), 128.3, 128.9 (2C), 129.4 (2C), 129.5 (4C), 130.0, 133.7, 133.8, 134.0, 144.5, 160.2, 177.0; IR (NaCl): $\tilde{\nu}$=1597 (s), 1509 (s), 1451 (s); HRMS (EI): *m/z* = 372.1287 (M^+), calc. for $C_{23}H_{20}N_2OS$ = 372.1296

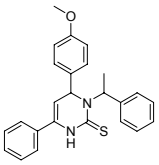


6-Isopropyl-3,4-diphenyl-3,4-dihydropyrimidine-2(1*H*)-thione (123c).
The product was isolated as a pale yellow oil. Yield: 59%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.19 (d, *J*=6.8 Hz, 6H, (C*H*$_3$)$_2$CH), 2.32–2.42 (m, 1H; (CH$_3$)$_2$C*H*), 4.90 (d, *J*=4.5 Hz, 1H; C*H*C), 5.17 (d, *J*=4.5 Hz, 1H; C*H*N), 6.94–6.96 (m, 2H; Ph-*H*), 7.14–7.17 (m, 8H; Ph-*H*), 7.60 (s, 1H; N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=20.1, 20.3, 30.4, 66.0, 98.0, 127.5 (2C), 127.7, 128.3 (3C), 128.6 (2C), 128.9 (2C), 139.4, 141.8, 144.0, 176.4; IR (NaCl): $\tilde{\nu}$=1698 (s), 1507 (s), 1456 (s), 1243 (s); HRMS (EI): *m/z* = 308.1338 (M^+), calc. for $C_{19}H_{20}N_2S$ = 308.1347.



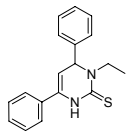
3-Ethyl-4-(4-methoxyphenyl)-6-phenyl-3,4-dihydropyrimidine-2(1*H*)-thione (**123d**).

The product was isolated as a pale yellow oil. Yield: 70%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.28 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 3.32–3.40 (m, 1H; C*H*$_2$), 3.84 (s, 3H; OC*H*$_3$), 4.35–4.44 (m, 1H; C*H*$_2$), 5.15–5.24 (m, 2H; C*H*C and C*H*N), 6.93 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.28–7.31 (m, 2H; Ph-*H*), 7.38–7.47 (m, 5H; Ph-*H*), 7.59 (s, 1H, N*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=11.6, 46.6, 55.3, 60.9, 100.5, 114.5 (2C), 125.0 (2C), 128.1 (2C), 128.9 (2C), 129.4, 132.9, 133.2, 134.1, 159.7, 175.3; IR (NaCl): $\tilde{\nu}$=1510 (s), 1462 (s), 1237 (s); HRMS (EI): *m/z* = 324.1290 (M^+), calc. for $C_{19}H_{20}N_2OS$ = 324.1294.



4-(4-Methoxyphenyl)-6-phenyl-3-(1-phenylethyl)-3,4-dihydropyrimidine-2(1*H*)-thione (**123e**).

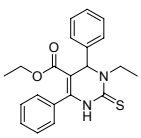
Product isolated as a pale yellow oil in a 5:6 mixture of diastereomers. Yield: 55%; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.20 (d, *J*=8.6 Hz, 3H; C*H*$_3$CH), 1.65 (d, *J*=7.0 Hz, 3H; C*H*$_3$CH) 3.60 (s, 3H; OC*H*$_3$), 3.72 (s, 3H; OC*H*$_3$), 4.62 (d, *J*=6.1 Hz, 1H; C*H*N), 4.87 (d, *J*=6.2 Hz, 1H; C*H*N), 5.09 (dd, *J*=2.4 Hz, *J*=6.1 Hz, 1H; C*H*C), 5.23 (dd, *J*=2.4 Hz, *J*=6.2 Hz, 1H; C*H*C), 6.39 (d, *J*=8.7 Hz, 2H; Ph-*H*), 6.63 (d, *J*=8.7 Hz, 2H; Ph-*H*), 6.78–7.32 (m, 26H; Ph-*H* and C*H*CH$_3$), 7.75 (s, 1H; N*H*), 7.80 (s, 1H, N*H*); ^{13}C NMR (400.13 MHz, $CDCl_3$,): δ=15.9, 17.3, 29.7 (2C), 55.2, 55.3, 56.0 (2C), 58.9, 59.6, 101.6, 101.7, 113.6, 114.4, 125.0 (2C), 125.2 (2C), 127.0 (2C), 127.2 (2C), 127.6 (4C), 127.9 (2C), 128.5 (2C), 128.7 (2C), 128.9 (4C), 129.3 (2C), 132.9, 133.0 (2C), 133.5, 135.2, 136.2, 138.6, 139.3, 158.5, 159.4, 177.3, 177.4; IR (NaCl): $\tilde{\nu}$=1510 (s), 1443 (s), 1249 (s); HRMS (EI): *m/z* = 400.1611 (M^+), calc. for $C_{25}H_{24}N_2OS$ = 400.1609.



3-Ethyl-4,6-diphenyl-3,4-dihydropyrimidine-2(1*H*)-thione (**123f**)

The product was isolated as a pale yellow oil. Yield: 72%; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=1.22 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 3.26–3.34 (m, 1H; C*H*$_2$), 4.37–4.45 (m, 1H; C*H*$_2$), 5.18–5.21 (m, 2H; C*H*C and C*H*N), 7.35–7.67 (m, 10H; Ph-*H*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=11.6, 46.8, 61.5, 100.3, 125.1 (2C), 126.7 (2C), 128.4, 128.9 (2C), 129.2 (2C), 129.4, 133.1, 141.9, 175.6; IR (NaCl): $\tilde{\nu}$=2973 (w), 2928 (w), 1520 (s), 1464 (s), 1287 (s), 1124 (s); HRMS (EI): *m/z* = 294.1187 (M^+), calc. for $C_{18}H_{18}N_2S$ = 294.1191.

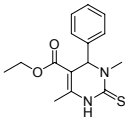
Method B (for compounds **125g–j**): In a 5 mL Pyrex microwave vial a sample of the corresponding thiazine **125g–j** (0.30 mmol) was dissolved in 1.5 mL of dry NMP. The vial was sealed and subjected to MW irradiation at 200°C for 35 min. After the reaction was completed the dark-colored solution was poured onto ice-water and stirred for 5-10 min. The solid precipitate was filtered and dried to give products **123g–j** as solids. For the rearrangement of **125j** an extractive work-up with diethyl ether and subsequent dry-flash chromatography (petroleum ether/EtOAc (1:1)) of the residue was performed to obtain product **123j**. Analytically pure samples were prepared by recrystallisation from appropriate solvents.



Ethyl 1-ethyl-1,2,3,6-tetrahydro-4,6-diphenyl-2-thioxopyrimidine-5-carboxylate (**123g**).

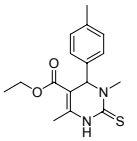
Yield: 88%; m.p. 167–169°C [*i*-propanol]; ^{1}H NMR (360 MHz, $CDCl_3$) δ=0.85 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 1.26 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 3.44 (sextet, *J*=7.2 Hz, 1H; C*H*$_2$N), 3.82–3.96 (m, 2H; OC*H*$_2$), 4.38 (sextet, *J*=7.2 Hz, 1H; C*H*$_2$N), 5.95 (s, 1H; C*H*N), 7.31–7.44 (m, 10H; Ph-*H*), 7.63 (s, 1H; N*H*); ^{13}C NMR (90 MHz, $CDCl_3$) δ=11.9, 13.5,

47.7, 60.3, 60.6, 102.8, 126.9 (2C), 128.3 (4C), 128.4, 129.0 (2C), 129.9, 134.0, 141.1, 143.8, 165.0, 174.5; MS (pos APCI) *m/z* 367 (28, M+1), 366 (100, M), 334 (53, M–32), 279 (43, M–87). Anal. Calcd for $C_{21}H_{22}N_2O_2S$: C, 68.82; H, 6.05; N, 7.64. Found: C, 68.50; H, 6.01; N, 7.45.



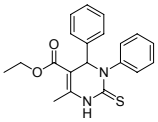
Ethyl 1,2,3,6-tetrahydro-1,4-dimethyl-6-phenyl-2-thioxopyrimidine-5-carboxylate (**123h**).

Yield: 95 %; m.p. 163–165°C [EtOH]; ^{1}H NMR (360 MHz, $CDCl_3$) δ=1.26 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.33 (s, 3H; C*H*$_3$), 3.30 (s, 3H, C*H*$_3$), 4.06–4.20 (m, 2H, C*H*$_2$), 5.39 (s, 1H; C*H*N), 7.28–7.34 (m, 5H, Ph-*H*), 8.09 (s, 1H; N*H*); ^{13}C NMR (90 MHz, $CDCl_3$) δ=14.2, 18.3, 40.4, 60.4, 63.3, 102.4, 126.9 (2C), 128.4, 128.9 (2C), 140.4, 142.5, 165.3, 175.0; MS (pos APCI) *m/z* 291 (19, M+1), 290 (100, M), 258 (43, M–32). Anal. Calcd for $C_{15}H_{18}N_2O_2S$: C, 62.04; H, 6.25; N, 9.65. Found: C, 62.12; H, 6.13; N, 9.52.



Ethyl 1,2,3,6-tetrahydro-1,4-dimethyl-2-thioxo-6-(*p*-tolyl)-pyrimidine-5-carboxylate (**123i**).

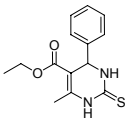
Yield: 95 %; m.p. 135–137°C [EtOH]; ^{1}H NMR (360 MHz, $CDCl_3$) δ=1.25 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.33 (s, 6H, C*H*$_3$N, C*H*$_3$C), 3.31 (s, 3H; C*H*$_3$Ph), 4.07–4.21 (m, 2H; C*H*$_2$), 5.36 (s, 1H; C*H*N), 7.13 (d, *J*=8.0 Hz, 2H; Ph-*H*), 7.19 (d, *J*=8.1 Hz, 2H; Ph-*H*), 7.79 (s, 1H; N*H*); MS (pos APCI) *m/z* 305 (14, M+1), 304 (100, M), 272 (48, M–32), 231 (42, M–73).



Ethyl 1,2,3,6-tetrahydro-4-methyl-1,6-diphenyl-2-thioxopyrimidine-5-carboxylate (**123j**).

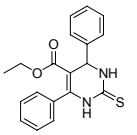
Yield: 61%; m.p. 171–172°C [EtOH]; ^{1}H NMR (360 MHz, $CDCl_3$) δ=1.21 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.43 (s, 3H; C*H*$_3$C), 4.05–4.20 (m, 2H; C*H*$_2$), 5.59 (s, 1H; CH*N*), 7.01–7.03 (m, 2H; Ph-*H*), 7.28–7.34 (m, 8H; Ph-*H*), 8.37 (s, 1H; N*H*); ^{13}C NMR (90 MHz, $CDCl_3$) δ=14.1, 18.3, 60.5, 65.2, 103.8, 127.4 (2C), 127.9 (2C), 128.2, 128.4, 128.7 (2C), 129.2 (2C), 141.1, 142.7, 143.8, 165.1, 176.1; MS (pos APCI) *m/z* 352 (31, M+1), 353 (100, M), 320 (33, M–32). Anal. Calcd for $C_{20}H_{20}N_2O_2S$: C, 68.16; H, 5.72; N, 7.95. Found: C, 67.84; H, 5.82; N, 7.86.

Method C (for **125k**,**l**). In a 5 mL pyrex microwave vial a sample of the corresponding thiazine (0.30 mmol) was dissolved in 1.5 mL of dry toluene. After the addition of a passive heating element[31] the vial was sealed and subjected to microwave irradiation at 210°C for 40 min. After the reaction was completed, the solvent was removed under reduced pressure and the solid crude product recrystallised from an appropriate solvent.



Ethyl 1,2,3,4-tetrahydro-6-methyl-4-phenyl-2-thioxopyrimidine-5-carboxylate (**123k**).

Yield: 67%; m.p. 200–202°C [acetonitrile] (lit. m.p. 201°C[32]); ^{1}H NMR (360 MHz, DMSO-d$_6$) δ=1.10 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.29 (s, 3H; C*H*$_3$C), 4.01 (q, *J* = 7.1 Hz, 2H; C*H*$_2$), 5.17 (d, *J*=3.4 Hz, 1H; C*H*N), 7.21 (d, *J*=7.2 Hz, 2H; Ph-*H*), 7.27 (t, *J*=7.3 Hz, 1H; Ph-*H*), 7.34 (t, J=7.30 Hz, 2H; Ph-*H*), 9.64 (s,1H; N*H*), 10.32 (s, 1H, N*H*).



Ethyl 1,2,3,4-tetrahydro-4,6-diphenyl-2-thioxopyrimidine-5-carboxylate (**123l**).

Yield: 63%; m.p. 191–193 °C [EtOH] (lit. m.p. 192°C[33]); ^{1}H NMR (360 MHz, DMSO-d$_6$) δ=0.72 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 3.74 (q, *J* = 7.1 Hz, 2H; C*H*$_2$), 5.27 (d,

J=3.6 Hz, 1H; C*H*N), 7.29–7.45 (m, 10H; Ph-*H*), 9.76 (s, 1H; N*H*), 10.48 (s, 1H; N*H*); ^{13}C NMR (90 MHz, DMSO-d_6) δ=13.8, 54.6, 59.9, 102.3, 126.9 (2C), 128.2 (2C), 128.3, 129.2 (4C), 129.6, 134.5, 143.5, 146.3, 165.4, 175.0; MS (pos APCI) *m/z* 339 (25, M+1), 338 (100, M), 304 (10, M–34), 279 (20, M–59).

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5

A Mild Chemo-Enzymatic Oxidation-Hydrocyanation Protocol

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Oxidation-hydrocyanation of γ,δ-unsaturated alcohols using (immobilised) TEMPO/PhI(OAc)$_2$ in combination with *Hb*HNL proceeds smoothly. After (*in situ*) protection, the resulting cyanohydrin derivatives were obtained in good overall yields and high *ee*'s. A mild TEMPO-catalysed oxidation protocol is described that yields β,γ-unsaturated aldehydes without isomerisation of the double bond and that is compatible with a subsequent *Hb*HNL-catalysed hydrocyanation performed in the same solvent.

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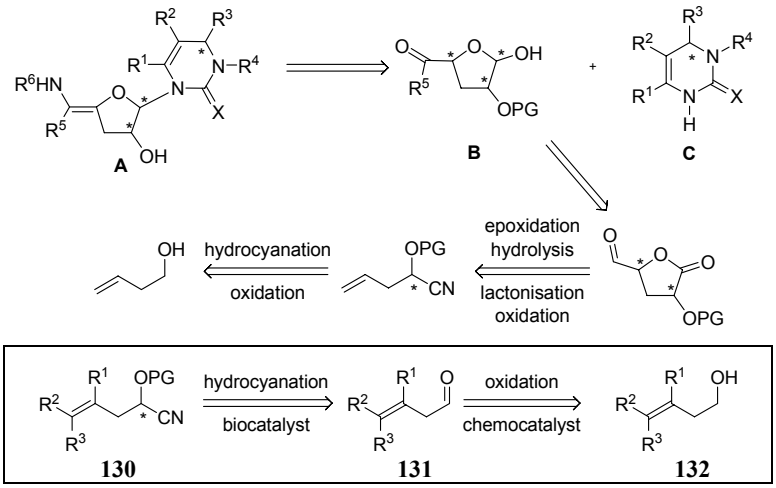
5.1 Introduction

Cyanohydrins are versatile chiral building blocks,[1–3] which can be transformed into α-aminonitriles,[4–6] α-hydroxyesters,[7] amino alcohols,[8] 2,3-disubstituted piperidines,[9] and 3-hydroxytetrahydropyridines.[10] The cyanohydrins are conveniently accessed by hydroxynitrile lyase (HNL) catalysed hydrocyanation of the corresponding aldehydes.[11] These biocatalysts are well established and both the (*R*)- and (*S*)-enantiomer of the cyanohydrins can be prepared in high yields and *ee* values.[12–14] During our studies towards a chemoenzymatic *de novo* synthesis of non-natural nucleosides (**A**) containing a 3'-deoxy ribose moiety we required a series of differently substituted, optically pure, γ,δ-unsaturated cyanohydrins **130** (Scheme 1). For this, a biocatalytic hydrocyanation of β,γ-unsaturated aldehydes **131** was identified as a key-step.



Scheme 5.1 Retrosynthesis of non-natural nucleosides containing a 3'-deoxyribose moiety

Oxidation of γ,δ-unsaturated primary alcohols **132** to the corresponding aldehydes **131** is the most direct way to prepare the required substrates for the HNL-catalysed hydrocyanation. However, many of the most widely employed oxidations like, *e.g.,* Swern-oxidation or chromium(VI)-based reagents (PDC, PCC, etc) require relatively alkaline conditions. Such conditions are not suitable for the transformation of primary alcohols **132** to yield the desired β,γ-unsaturated aldehydes **131** because they promote the isomerisation of the double bond and thus yield the α,β-unsaturated isomers, which are difficult to separate from the required product.[15-19] Furthermore, the alkaline conditions would also promote the racemic chemical addition of HCN to the aldehydes **131** in the follow-up reaction, a reaction that should result in the desired enantiopure cyanohydrin derivatives **130**.

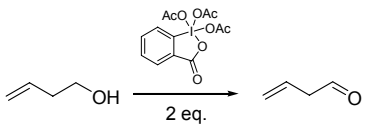
The work described in this chapter aims at a mild oxidation–hydrocyanation protocol to produce optically pure γ,δ-unsaturated (*S*)-cyanohydrins **130** starting from readily available primary alcohols **132**. Since enzymatic redox reactions tend to be difficult due to problems with co-factor regeneration, a chemical reagent is envisaged for the oxidation step. The enantioselective C-C bond formation under close to neutral conditions, however, is difficult to perform with a chemical catalyst, therefore the efficient and highly enantioselective *Hb*HNL will be employed.[20]

(*R*)-Cyanohydrins are accessible in good yields and *ee*'s using the hydroxynitrile lyase *Prunus amygdalus* (*Pa*HNL) from almonds, which catalyses the hydrocyanation efficiently.[12] In general, (*S*)-cyanohydrins are readily available using the hydroxynitrile lyase from the rubber tree *Hevea brasiliensis* (*Hb*HNL).[13,14,20-27] However, reports on the enantioselective synthesis of γ,δ-unsaturated cyanohydrins **130** from β,γ-unsaturated aldehydes **131** are scarce.[15,28] Only two examples are known of (*R*)-**130** produced by *Pa*HNL, while no examples exist for the preparation of (*S*)-**130** using (*S*)-selective enzymes such as *Hb*HNL.

5.2 Results and Discussion

5.2.1 Homogeneous oxidation

In initial experiments, the application of the very mild Dess–Martin periodinane (DMP) to oxidise **132a** (Scheme 5.2) without isomerisation seemed feasible but following this procedure, we encountered a couple of practical disadvantages.[29,30] Commercial DMP is relatively expensive while at the same time its preparation is not entirely trivial and its storage can lead to degradation.[31] When oxidising primary alcohols of relatively low molecular weight, a large amount of DMP is needed to convert the alcohol to the aldehyde.

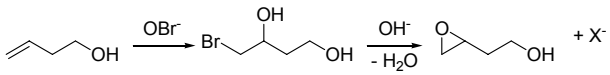


132a **131a**

Scheme 5.2 DMP-oxidation of 3-butenol

In our case, one gram (13.9 mmol) of alcohol **132a** is converted to the corresponding aldehyde **131a** almost quantitatively, but this can only be achieved by using twelve grams (28.3 mmol) of DMP. Thus the Dess–Martin oxidation suffers from poor atom efficiency and the reaction work-up is troublesome. Consequently, the use of DMP in larger scale production of β,γ-unsaturated aldehydes becomes less convenient. Furthermore, we found

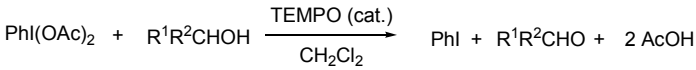
that the filtration/evaporation procedure, which is necessary after the Dess–Martin oxidation, was accompanied by some isomerisation of the product aldehyde. Therefore we turned to another method to oxidise alcohols **132** to the corresponding aldehydes **131**. The use of relatively stable organic nitroxyl radicals, like 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), as catalysts for the mild oxidation of alcohols has found widespread application.[32,33] A range of different procedures is known using TEMPO, *e.g.* in combination with p-TsOH, oxygen and CuCl, KBr and sodium hypochlorite (NaOCl) or a hypervalent iodine species.[34] The first procedure is an acid-promoted disproportionation using 2 equiv. TsOH and TEMPO, which makes it not a very elegant method for the oxidation of small molecules. Like the oxidation with DMP, large amounts of oxidant are necessary. The second approach, oxidation using oxygen and CuCl, is usually performed in DMF and is therefore not applicable for the synthesis of very volatile aldehydes. The oxidation with KBr and NaOCl was at first sight interesting to try in the oxidation of 3-butenol, because it uses simple chemicals and is very mild. However not the alcohol was oxidised, but the double bond was epoxidised. The mechanism of this epoxidation can be envisioned as depicted in Scheme 5.3. NaOCl and KBr form in situ the highly reactive hypobromide, which adds to the double bond and results in epoxide **134**.[35]



132a **133** **134**

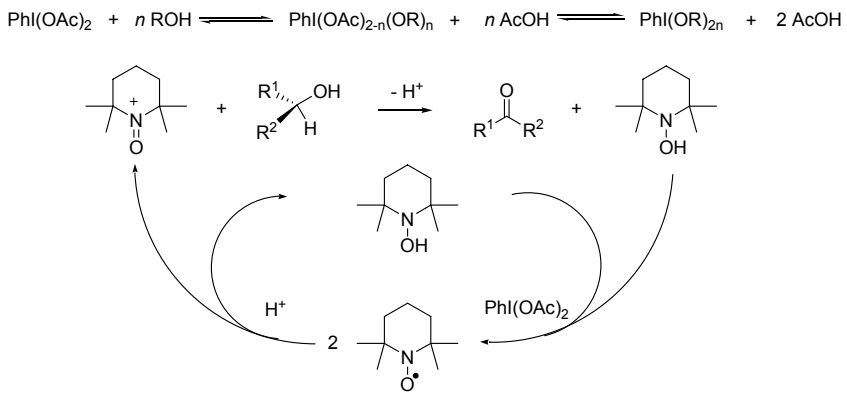
Scheme 5.3 Formation of epoxide **134** in NaOCl/KBr/TEMPO reaction

Finally the oxidation of sensitive primary alcohols that employs a catalytic amount of TEMPO together with hypervalent iodine species, $PhI(OAc)_2$, was investigated (Scheme 5.4).



Scheme 5.4 Oxidation of alcohols with $PhI(OAc)_2$/TEMPO

In this reaction an equilibrium between the alcohol (ROH) and $PhI(OAc)_2$ is responsible for the exchange of one or both acetate ligands around the iodine atom (OR). The acetic acid that is formed catalyses dismutation of TEMPO to hydroxyl amine and the corresponding oxoammonium salt. The latter species is responsible for the selective oxidation of the primary alcohol to the aldehyde while being reduced to hydroxyl amine. Then $PhI(OAc)_2$ completes the catalytic cycle via regeneration of TEMPO (Scheme 5.5).[34]



Scheme 5.5 Proposed reaction pathway for the oxidation of alcohols using $PhI(OAc)_2$/TEMPO.

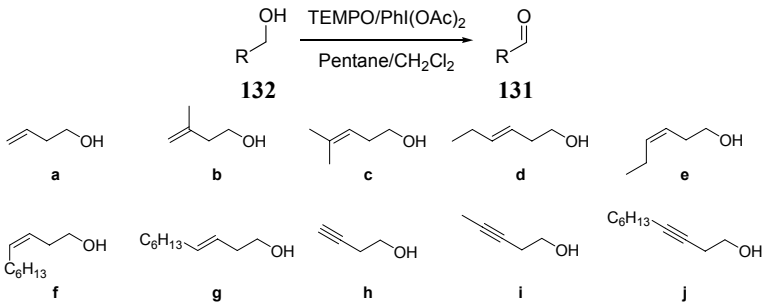
Initially the reaction was performed following the literature procedure[34] by using a 0.2 M solution of **132a** in CH_2Cl_2, 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO (entry 1,Table 5.1). After 2.5 h only 15% had been converted to the desired β,γ-unsaturated aldehyde **131a**. Although more oxidising agent improved the conversion, still only an unsatisfactory 38 or 50 % conversion (entries 2 and 3,Table 5.1) was detected after 2.5 h. Prolonged reaction times did not result in a better conversion but instead isomerisation to the undesired α,β-unsaturated aldehyde occurred. Instead of using CH_2Cl_2, an attempt was made to change the solvent to a more environmentally friendly mixture of pentane and CH_2Cl_2. This also has the advantage that this mixture is more suitable for *Hb*HNL.[24] To our satisfaction, application of **132a** dissolved in a 9:1 mixture of pentane and CH_2Cl_2, respectively, together with 1.1 equiv. of $PhI(OAc)_2$, and 0.1 equiv. of TEMPO gave complete and selective conversion to **131a** after 2.5 h (entry 4, Table 5.1).

Table 5.1 Oxidation of **132**a with TEMPO/$PhI(OAc)_2$

Entry	$PhI(OAc)_2$ (equiv.)	TEMPO (equiv.)	Solvent	Conversion[a] (%)
1	1.1	0.1	CH_2Cl_2	15
2	1.1	0.2	CH_2Cl_2	38
3	1.2	0.2	CH_2Cl_2	50
4	1.1	0.1	Pentane/CH_2Cl_2 (9:1)	100

a) Conversion of **3a** after 2.5h at room temperature determined by ^{1}H NMR spectroscopy.

A number of γ,δ-unsaturated primary alcohols **132** were treated under similar conditions (Scheme 5.6 and Table 5.2). Conditions were optimised towards a minimum degree of isomerisation and optimum conversion. As can be seen, homo-allylic primary alcohols **132a**–**g** were readily converted to the corresponding β,γ-olefinic aldehydes **131a**–**g**. Only one of three homo-propargylic alcohols, **132i**, could be oxidised with TEMPO/PhI(OAc)$_2$, while the other two react sluggishly (**132h** and **j**).



Scheme 5.6 Oxidation of γ,δ-unsaturated alcohols to β,γ-unsaturated aldehydes

It should be noted that the above TEMPO/PhI(OAc)$_2$ oxidation protocol encountered some problems during reaction work-up. Even when the aldehyde was carefully co-distilled with diethyl ether under reduced pressure, a significant isomerisation of the aldehyde was observed. This provided an additional incentive to develop the planned reaction protocol.

Table 5.2 Optimised TEMPO/PhI(OAc)$_2$-oxidation of γ,δ-unsaturated alcohols **132**

Substrate	Mmol **132a-132j**	Conv.[a] (%)	PhI(OAc)$_2$ (equiv.)	TEMPO (equiv.)	Pentane: CH_2Cl_2	Conc. (mM)	Reaction time (min.)
132a	27.5	quant.	1.1	0.1	9:1	0.20	150
132b	5.00	quant.	1.1	0.1	9:2	0.36	120
132c	2.00	quant.	1.1	0.1	9:2	0.36	40
132d	2.00	quant.	1.1	0.1	9:2	0.36	100
132e	2.00	quant.	1.1	0.1	9:2	0.36	100
132f	4.00	quant.	1.2	0.2	2:1	0.66	30
132g	4.00	quant.	1.2	0.2	2:1	0.66	30
132h	0.25	0	1.2	0.2	1:1	1	--
132i	0.50	41	1.8	0.2	3:2	1	70
132j	1.00	0[b]	1.2	0.2	1:1	1	150

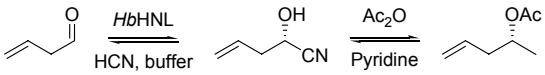
a) Conversion of **132** determined by ^{1}H NMR; b) Different concentrations, amounts of chemicals, and temperatures were tried, however oxidation was never observed.

However, during the oxidation two equivalents of acetic acid are formed. As this acid would cause an immediate deactivation of the *Hb*HNL it had to be removed prior to the hydrocyanation reaction.[36] By washing the reaction mixture at the end of the TEMPO/PhI(OAc)$_2$ oxidation with a saturated solution of NaHCO$_3$, all the acetic acid was removed completely. To our satisfaction no isomerisation was detected.

5.2.2 Homogeneous HNL-catalysed hydrocyanation of β,γ-unsaturated aldehydes

Since both the formation of cyanohydrins and the isomerisation of the aldehyde **131a** are base-catalysed, the enzyme reaction should be performed in a mildly acidic buffer. A direct transfer of the conditions that were used in the *Pa*HNL-catalysed synthesis of the (*R*)-enantiomer of **130a** is not possible. *Pa*HNL and *Hb*HNL are structurally not related and their optimum reaction conditions are different.[37] Initially two different pH's (4.0 and 5.0) and two different temperatures (0° and 25° C) were investigated for the *Hb*HNL catalysed addition of HCN to the *in situ* generated aldehyde **131a** following a general procedure.[24] The results are summarised in Table 5.3.

Table 5.3 Selectivity at complete conversion of **131a** in the *Hb*HNL-catalysed hydrocyanation; various pH values and temperatures.[a]



131a **130a** **135a**

Entry	pH	Temp. (°C)	*ee* of **135a**
1	4.0	0	82
2	5.0	0	71
3	5.0	25	66

a) Initially, *Hb*HNL and 3 equiv. of HCN were used but after 30 min the reaction stopped. Complete conversion was only achieved by the addition of extra *Hb*HNL and 1.5 equiv HCN (see experimental section).

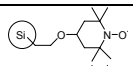
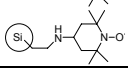
When the enzymatic hydrocyanation was performed at 0 °C and pH 4.0 the desired γ,δ-unsaturated cyanohydrin **130a** was formed with reasonable enantioselectivity (*ee* = 82 % entry 1, Table 5.3). Even though this result is in line with *ee*'s obtained previously with other short-chain aldehydes[1] it is still insufficient for synthetic use. The relatively low *ee* is not caused by chemical background reaction, as this is virtually absent under these conditions. Therefore, other reasons were investigated.

The *Hb*HNL catalysed hydrocyanation of **131a** as described above stopped after 30 min (Table 5.3). Apparently the enzyme was deactivated and additional *Hb*HNL and HCN were needed to allow the reaction to complete. After $NaHCO_3$ neutralisation of the crude reaction mixture that results from the oxidation, part of the highly reactive TEMPO is still present. To minimise a potentially harmful effect of TEMPO on the activity and the selectivity of the *Hb*HNL we investigated the influence of different immobilised variants of TEMPO in the oxidation-hydrocyanation protocol. This should allow for efficient removal of TEMPO from the reaction mixture just before addition of the *Hb*HNL. Furthermore, such a procedure opens the way to a more atom-efficient protocol that enables recycling of the TEMPO-catalyst.

5.2.3 Heterogeneous oxidation

TEMPO was immobilised on colloidal silica according to a known procedure[38] and compared with commercial TEMPO immobilised on silica gel. The TEMPO immobilisates were screened in the oxidation of **132a** for optimal conversion and minimal isomerisation of the aldehyde product **131a** (Table 5.4). The data in entry 1 of table 5.4 show that oxidation of **132a** using TEMPO immobilised on colloidal silica proceeds about three times faster as compared to oxidation of **132a** using soluble TEMPO (Table 5.2). After 40 min at room temperature the reaction was complete and no isomerisation was observed. The TEMPO on colloidal silica could easily be recovered (by filtration) and re-used at least once without any loss of activity. The commercial TEMPO on silica (entry 2, Table 5.4) gave, under similar reaction conditions, only a few percent of 3-butenal **131a**. In the ^{1}H NMR spectrum of the reaction mixture, the unreacted alcohol **132a** was clearly identified together with signals that could only be attributed to the undesired isomer 2-butenal (crotonaldehyde).

Table 5.4 Oxidation of **132a** using TEMPO immobilised on colloidal silica and commercial TEMPO immobilised on silica.[a]

Entry	Structure	Loading (mmol/g)	Equiv.	**131a** (%)[b]	Reaction time
1[c]		0.27	0.16	>98	40 min.
2[d]		0.61	0.35	0[e]	3.5 h

a) 1.2 equiv of $PhI(OAc)_2$ in a 0.2 M solution of **132a** in a 9:1 pentane/CH_2Cl_2 mixture at room temperature; b) Conversion determined by ^{1}H NMR spectroscopy; c) TEMPO immobilised on colloidal silica; d) Commercial TEMPO immobilised on silica, available from Sigma-Aldrich; e) The reaction yielded the unwanted isomer of **131a**, crotonaldehyde.

5.2.4 Heterogeneous HNL-catalysed hydrocyanation of β,γ-unsaturated aldehydes

Finally, the oxidation of **132a** catalysed by immobilised TEMPO was combined with the *Hb*HNL catalysed hydrocyanation to arrive at the desired cyanohydrin **130a**.[39] After the oxidation reaction, the immobilised TEMPO was removed by filtration and the resulting mixture neutralised with a saturated $NaHCO_3$ solution. The resulting solution is directly used in the *Hb*HNL catalysed formation of γ,δ-unsaturated **130a**. Compound **130a** (*ee* = 93 %; entry 1, Table 5.5) could be isolated as its TBDMS-ether **5a** in 37 % overall yield[40] starting from the γ,δ-unsaturated primary alcohol **132a**. Although, under these optimised conditions, the isolated yield of **130a** is 5-10% lower compared to the yield obtained after the oxidation-hydrocyanation sequence using soluble TEMPO, the optical purity of **130a** is considerably higher (93% *versus* 82%; entry 1, Table 5.5 *versus* entry 1, Table 5.3) when performing this protocol using immobilised TEMPO. The results indicate that TEMPO has a negative effect both on the activity and the selectivity of the enzyme.

The above procedures were used to prepare γ,δ-unsaturated cyanohydrins **130b**–**e**, starting from the corresponding primary alcohols **132b**–**e**. The resulting cyanohydrins were directly protected as acetates. The results are summarised in Table 5.5. A similar trend with regard to isolated yield and optical purity that was observed for the synthesis of **5a** is also found for the preparation of **135b**–**e**. In general, the *ee*'s of **135b**–**e** (Table 5.5) are significantly higher whereas their isolated yields (calculated from **132b**–**e**) are somewhat lower if they are prepared by the immobilised TEMPO/*Hb*HNL protocol compared to the *ee*'s and yields obtained by applying the same protocol by using soluble TEMPO. As the reactions were performed on a 100 mg scale and the compounds involved in this reaction are relatively polar and/or volatile some loss/low yields could not be avoided.

When testing both pH 4.0 and 5.0 for aldehydes **131c**–**e** pH 5.0 proved to be favourable and was therefore utilised. Finally, the γ,δ-unsaturated primary alcohols **132f** and **132g** were readily oxidised by the TEMPO/$PhI(OAc)_2$ procedure to the corresponding aldehydes **131f** and **131g**. However, both aldehydes proved unreactive towards *Hb*HNL catalysed hydrocyanation. This can be attributed to the length of the alkyl chain, which is known to be detrimental to *Hb*HNL activity.[11–14]

Table 5.5 The three-step protocol oxidation-hydrocyanation and protection reaction starting from **132a**–**e** using both homogeneous and heterogeneous TEMPO in combination with *Hb*HNL.

Substrate	Product	pH	*ee* (%) Homogenous TEMPO[b]	Yield[a] (%)	*ee* (%) Heterogenous TEMPO[b]	Yield[a] (%)
132a	**136a**	4.0	82	n.d	93	37
132b	**135b**	4.0	92	54	97	43
132b	**135b**	5.0	95	43	92	37
132c	**135c**	5.0	78	48	95	23
132d	**135d**	5.0	61	52	87	26
132e	**135e**	5.0	72	51	91	7[c]

a) Isolated yields over 3 steps; b) *ee* determined by chiral GC; c) very low yield due to isolation problems.

5.3 Conclusions

In summary, we have developed an efficient oxidation-hydrocyanation protocol that produces optically enriched γ,δ-unsaturated cyanohydrins in good yields starting from the corresponding primary alcohols. The oxidation of the alcohols with TEMPO and $PhI(OAc)_2$ is a mild and selective method to prepare β,γ-unsaturated aldehydes, which are otherwise difficult to access as they readily undergo isomerisation to the α,β-unsaturated analogues. The thus generated aldehydes can be used directly in the subsequent *Hb*HNL-catalysed hydrocyanation to give the desired optically enriched γ,δ-unsaturated cyanohydrins. Moreover, when the *Hb*HNL catalyst was used in combination with the TEMPO catalyst immobilised on colloidal silica *ee*'s up to 97% and overall yields up to 43% of the final cyanohydrin derivatives were obtained. The TEMPO-catalyst could be re-used at least once without any loss of catalytic activity.

5.4 Acknowledgements

We thank Dr. Marek Smoluch for measuring the HRMS samples and DSM (M. Wubbolts) for the *Hb*HNL.

5.5 Experimental section

5.5.1 General

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 (250.13 and 62.90, respectively) or a Bruker MSL 400 (400.13 and 100.61 MHz respectively) spectrometer; chemical shifts (δ) are given in ppm,

internally referenced to residual solvent resonances (^{1}H: δ 7.29 ppm, ^{13}C: δ 77.0 ppm). Column chromatography was performed on Baker 7024-02 silica gel (40 μ, 60 Å) with petroleum ether (PE, boiling range 40-60 °C) and ethyl acetate (EA) as eluents. Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC were visualised by UV-detection or 5% (w/v) aqueous $KMnO_4$. High-resolution mass spectra (HRMS, EI) and mass spectra (MS) were recorded on a Finnigan Mat 900 spectrometer at 70 eV. IR spectra were recorded on a Mattson 6030 Galaxy spectrophotometer and are reported in cm^{-1}. To follow the course of the reactions, samples were taken directly from the reaction mixtures, dissolved in $CDCl_3$ and analysed by ^{1}H NMR spectroscopy. For the oxidation and enzyme reaction, the conversion was determined by monitoring the H_2C-O (δ 3.59–3.76) and the *H*C=O (δ 9.66–9.74) signal respectively. The racemic cyanohydrin acetates were prepared from the corresponding aldehydes according to literature.[41] The enantiomeric excess of the acylated (*S*)-cyanohydrins **135** was determined on a Shimadzu GC-17A, equipped with a β-cyclodextrin column (CP-Chirasil-Dex CB 25m×0.32 mm ID), a FID detector, and a Shimadzu Auto-injector AOC-20i. The carrier gas was He with a linear gas velocity of 75 cm/s at 155kPa. The GC-retention times are summarised in Table 5.6. Optical rotations were measured on an AA-10 automatic polarimeter from Optical Activity Ltd. Pro analysis grade γ,δ-unsaturated primary alcohols **132a–e** and **3h–j** were all commercially available and used without purification except for 3-butenol **132a**, which was distilled and stored under nitrogen and over molecular sieves 4Å. Primary alcohols **132f** and **132g** were prepared by hydrogenation and LAH reduction, respectively, starting from 3-decynol following literature procedures.[42,43] TEMPO immobilised on silica gel (70-120 mesh) was purchased from Sigma-Aldrich. The loading of the TEMPO on colloidal silica was determined by elemental analysis on an Elementar Vario EL III analyzer. The hydroxynitrile lyase from *Hevea brasiliensis* (*Hb*HNL) was a generous gift from DSM (Wubbolts, NL). The activity of the *Hb*HNL (13.1 U/mg protein solution) was determined according to standard procedures.[44,45]

5.5.2 Synthetic Procedures and Physical Data

Colloidal silica: A mixture of tetramethoxysilane (25 mL) and acidic water (50 mL, pH adjusted to 2.8 by addition of HCl) was stirred until a homogeneous mixture was formed. After ageing at room temperature for 18 h, the gel was crushed to a fine powder and the water was removed by azeotropic distillation with toluene. The solid was collected by filtration and dried at 120ºC overnight to give a white glass like powder. The TEMPO was immobilised on the colloidal silica according to literature.[38]

Table 5.6 Temperature program and retention times for the GC analysis of acetates **135a–e**

Compound	Temperature (°C)	R_t (***R***) **135a–e** (min.)	R_t (***S***) **135a–e** (min.)
135a	100	3.13	4.16
135b	135	1.13	1.20
135c	135	1.61	1.81
135d	135	1.63	1.83
135e	135	1.67	1.91

General Oxidation-hydrocyanation procedure A, using homogeneous TEMPO and *Hb*HNL: To a solution of γ,δ-unsaturated primary alcohol **3a**–**e** in a pentane/CH_2Cl_2 mixture, $PhI(OAc)_2$ and TEMPO were added, all quantities are according to Table 5.2. The reaction mixture was stirred at room temperature until full conversion of the alcohol was reached (according to ^{1}H NMR). Then, saturated $NaHCO_3$ solution was added at 0°C to the reaction mixture until the CO_2 development ended. The aqueous layer was removed and hydroxynitrile lyase from *Hevea brasiliensis* (1.45 KU/mmol **132a**–**e**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0°C to generate a 1:1 mixture (v/v) of organic phase to buffer was added. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN dissolved in MTBE was added. [The HCN-solution was prepared by dissolving sodium cyanide (3.0 equiv.) in water (10 mL) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3×8 mL) at 0°C]. After the hydrocyanation was complete (according to ^{1}H NMR) the organic layer was isolated and dried over $MgSO_4$. In the cases where the emulsion was too stable it was extracted with CH_2Cl_2 (5–10 mL). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 mL/mmol substrate) and acetic anhydride (3 equiv.), pyridine (2 equiv.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **135b**–**e**. The reaction mixture was stirred overnight, washed with 1% HCl (2×10 mL), water (2×10 mL), followed by washing with saturated $NaHCO_3$ (2×10 mL) and water (2×10 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **130a**, starting from 6 mmol, only an analytical sample was derivatised in the same manner. This showed an *ee* of 82 %.

(2*S*)-2-acetoxy-4-methyl-4-pentenenitrile (135b)
The title compound was prepared from **132b** (430 mg, 5 mmol) according to general procedure A, using pH 4.0. (***S***)-**135b** was obtained as a clear oil (414 mg, 54% yield, 92% *ee*). For characterisation, see (***S***)-**135b** obtained from general procedure B.

(2*S*)-2-acetoxy-5-methyl-4-hexenenitrile (135c)
The title compound was prepared from **132c** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. (***S***)-**135c** was obtained as a clear oil (161 mg, 48% yield, 78% *ee*). For characterisation, see (***S***)-**135c** obtained from general procedure B.

(2*S*,4*E*)-2-acetoxy-4-heptenenitrile (135d)
The title compound was prepared from **132d** (200 mg, 2 mmol) according to general procedure A, using pH 5.0. (***S***)-**135d** was obtained as a clear oil (174 mg, 52% yield, 61% *ee*). For characterisation, see (***S***)-**135d** obtained from general procedure B.

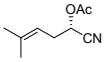
(2*S*,4*Z*)-2-acetoxy-4-heptenenitrile (135e)
The title compound was prepared from **132e** (200 mg, 2 mmol) according to general procedure A, using pH5.0. (***S***)-**135e** was obtained as a clear oil (173 mg, 51% yield, 72% *ee*). For characterisation, see (***S***)-**135e** obtained from general procedure B.

General Oxidation-hydrocyanation procedure B using TEMPO immobilisates and *Hb*HNL

A 0.2 M solution of γ,δ-unsaturated primary alcohols **132a–e** (1 equiv.) in pentane/CH_2Cl_2 (9:1) mixture, $PhI(OAc)_2$ (1.1 equiv.) was mixed with immobilised TEMPO (TEMPO on colloidal silica: 0.27 mmol/g, 0.16 equiv. or commercial TEMPO on silica gel: 0.61 mmol/g, 0.35 equiv.) and stirred at room temperature until completion (according to ^{1}H NMR). After filtration of immobilised TEMPO, a saturated $NaHCO_3$ solution was added to the reaction mixture at 0°C until the CO_2 development ended. The aqueous layer was removed and to the organic layer was added hydroxynitrile lyase from *Hevea brasiliensis* (1.45 /mmol **132a–e**) dissolved in an equivolume of 0.1 M citrate buffer (pH 4.0 or pH 5.0) at 0°C to generate a 1:1 mixture (v/v) of organic phase to buffer. The mixture was stirred vigorously until a stable emulsion was obtained, after which HCN (3 equiv.) dissolved in MTBE was added. [The HCN-solution was prepared by dissolving sodium cyanide (3.0 equiv.) in water (10 mL) and adjusting the pH of the solution to 4.8 by addition of citric acid. This aqueous solution was extracted with MTBE (3×8 mL) at 0°C]. After the hydrocyanation was complete (according to ^{1}H NMR) the organic layer was separated and dried over $MgSO_4$. In the cases where the emulsion was too stable it was extracted with CH_2Cl_2 (5–10 mL). After evaporation of the solvents, the resulting oil was dissolved in CH_2Cl_2 (2 mL/mmol substrate) and acetic anhydride (3 equiv.), pyridine (2 equiv.) and 4-DMAP were added to the solutions of (*S*)-cyanohydrins **130b–e**. The reaction mixture was stirred overnight, washed with 1% HCl (2×10 mL), water (2×10 mL), followed by washing with saturated $NaHCO_3$ (2×10 mL) and water (2×10 mL). The organic layer was dried over Na_2SO_4 and concentrated under vacuum. The crude product was purified by column chromatography. In the case of **130a**, only an analytical sample was derivatised in the same manner to give the acetate with an *ee* of 95 %. The remaining solution was derivatised as the corresponding TBDMS-ether **136a** (see below).

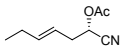
(2*S*)-2-acetoxy-4-methyl-4-pentenenitrile (135b)

The title compound was prepared from **132b** (431 mg, 5 mmol) according to general procedure B, using pH 4.0. **(*S*)-135b** was obtained as a clear oil (330 mg, 43% yield, 97% *ee*); R_f (PE/EA, 95:5)= 0.27; $[\alpha]^{22}_D$= –74 (*c*=1, $CHCl_3$); ^{1}H NMR (250.13 MHz): δ=1.83 (s, 3H; C*H*$_3$C═C), 2.16 (s, 3H; C*H*$_3$C═O), 2.64 (d, *J*=7.1 Hz, 2H; C*H*$_2$CHCN), 4.94 (s, 1H; *H*$_2$C═CCH$_3$), 5.02 (s, 1H; *H*$_2$C═CCH$_3$), 5.50 (t, *J*=7.1 Hz, 1H; C*H*CN); ^{13}C NMR (62.90 MHz): δ=20.3, 22.3, 40.4, 59.7, 116.10, 116.7, 137.6, 168.9; IR (NaCl): $\tilde{\nu}$= 2975 (m), 1756 (s), 1376 (s), 1221 (s), 1053 (s), 907 (m); MS ($C_8H_{11}O_2N$, m/z, relative intensity): 153 [M^+, 2], 111 (4), 93 (100), 66 (68), 55 (32);



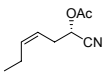
(2*S*)-2-acetoxy-5-methyl-4-hexenenitrile (135c)

The title compound was prepared from **132c** (75 mg, 0.75 mmol) according to general procedure B, using pH 5.0. **(*S*)-135c** was obtained as a clear oil (29 mg, 23% yield, 95% *ee*); R_f (PE/EA, 95:5) = 0.27; $[\alpha]^{22}_D$ = – 46 (*c*=1, $CHCl_3$); ^{1}H NMR (250.13 MHz): δ=1.71 (s, 3H; C*H*$_3$C═C), 1.79 (s, 3H; C*H*$_3$C═C), 2,18 (s, 3H; C*H*$_3$C═O), 2.61–2.67 (m, 2H; C*H*$_2$), 5.19 (t, *J*=7.2 Hz, 1H; C═C*H*), 5.30 (t, *J*=6.9 Hz, 1H; C*H*CN); ^{13}C NMR (100.61 MHz): δ=17.9, 20.3, 25.7, 31.1, 60.9, 114.9, 116.7, 138.5, 168.9; IR (NaCl): $\tilde{\nu}$= 2966 (m), 1755 (s), 1375 (m), 1220 (s), 1036 (s), 801 (s); MS ($C_9H_{13}O_2N$, m/z, relative intensity): 167 [M^+, 16], 149 (16), 142 (64), 113 (40), 95 (40), 69 (100), 55 (48).



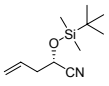
(2*S*,4*E*)-2-acetoxy-4-heptenenitrile (135d)

The title compound was prepared from **132d** (75 mg, 0.75 mmol) according to general procedure B, using pH 4.0. (***S*)-135d** was obtained as a clear oil (32.6 mg, 26% yield, 87% *ee*); R_f (PE/EA, 95:5) = 0.35; $[\alpha]^{22}_D$ = –32 (*c*=1, $CHCl_3$); ^{1}H NMR (250.13 MHz): δ=1.02 (t, *J*=7.4 Hz, 3H; C*H*$_3$), 2.03–2.12 (m, 2H; CH_3CH_2), 2.15 (s, 3H; CH_3C═O), 2.56–2.62 (m, 2H; C*H*$_2$CHCN), 5.30–5.45 (m, 2H; C*H*═C*H*), 5.71–5.82 (m, 1H; C*H*CN); ^{13}C NMR (100.61 MHz): δ=13.2, 20.1, 25.4, 35.4, 60.9, 116.4, 119.5, 139.0, 168.9; IR (NaCl): $\tilde{\nu}$= 2965 (m), 1755 (s), 1373 (m), 1222 (s), 1037 (s), 970 (m); MS ($C_9H_{13}O_2N$, m/z, relative intensity): 167 [M^+, 16], 149 (52), 142 (24), 106 (48), 83 (52), 69 (100);



(2*S*,4*Z*)-2-acetoxy-4-heptenenitrile (135e)

The title compound was prepared from **132d** (75 mg, 0.75 mmol) according to general procedure B, using pH 4.0. (***S*)-135d** was obtained as a clear oil (8.8 mg, 7% yield, 91% *ee*); Rf (PE/EA 95:5) = 0.35; $[\alpha]^{22}_D$ = – 46 (*c*=1, $CHCl_3$); ^{1}H NMR (250.13 MHz,): δ=1.02 (t, *J*=7.4 Hz, 3H; C*H*$_3$), 2.05–2.11 (m, 2H; CH_3CH_2), 2.15 (s, 3H; CH_3C═O), 2.64–2.69 (m, 2H; C*H*$_2$CHCN), 5.29–5.41 (m, 2H; C*H*═C*H*), 5.66–5.76 (m, 1H; C*H*CN); ^{13}C NMR (62.90 MHz): δ=13.3, 19.1, 20.3, 30.1, 59.8, 116.6, 118.8, 137.8, 169.0; IR (NaCl): 2964 (m), 1715 (s), 1558 (m), 1259 (s), 1211 (s), 800 (s); MS ($C_9H_{13}O_2N$, m/z, relative intensity): 168 [M^++1, 2], 142 (16), 106 (84), 80 (64), 69 (100).



(2*S*)-2-(*t*-butyldimethylsilanyloxy)-4-pentenenitrile (136a)

A solution of recrystallised imidazole (0.87 g, 12.8 mmol) and *t*-butyldimethylsilylchloride (2.11 g, 14 mmol) in 70 mL DMF was stirred at 0°C for 20 min. The crude solution of **130a**, prepared from **132a** (844 mg, 11.7 mmol) according to general procedure B using pH 4 was added, the mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted with 70 mL of water and extracted with diethyl ether (3×110 mL). The combined organic layers were washed with water (2×100 mL) and then with brine (1×100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by column chromatography on silica (PE/EA, 98:2) yielding **5a** as a clear oil (659 mg, 37% yield, 95% ee); R_f (PE/EA, 95:5) = 0.74; $[\alpha]^{22}_D$ = –60 (*c*=1, $CHCl_3$); ^{1}H NMR (250 MHz): δ=0.17 (s, 3H; C*H*$_3$Si), 0.22 (s, 3H; C*H*$_3$Si), 0.94 (s, 9H; (C*H*$_3$)$_3$C), 2.53–2.59 (m, 2H; C*H*$_2$CHCN), 4.47 (t, *J*=6.5 Hz, 1H; C*H*CN), 5.23–5.30 (m, 2H; CH═C*H*$_2$), 5.84 (ddt, *J*=17.4 Hz, *J*=9.8 Hz and *J*=7.0 Hz, 1H; C*H*=CH$_2$); ^{13}C NMR (62.90 MHz): δ= -4.9, -4.7, 18.5, 25.9 (3C), 41.1, 62.3, 120.0, 120.5, 131.4; IR (NaCl): $\tilde{\nu}$= 2956 (s), 2860 (s), 1652 (m), 1558 (m), 1259 w), 1114 (m); HRMS (EI): *m/z* = 211.1392 (M^+), calc. for $C_{11}H_{21}NOSi$ = 211.1392; MS ($C_{11}H_{21}NOSi$, m/z, relative intensity): 210 [M^+, 15], 156 (66), 126 (100), 73 (74).

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6

Synthetic Studies towards the 3′-Deoxyribose Moiety

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A chemical study has been performed on the synthesis of a lactone precursor of 3'-deoxy-5'-hexylribose. Starting from a simple homopropargylic alcohol via oxidation, hydrocyanation, reduction, epoxidation and finally hydrolysis-induced lactonisation a 3'-deoxy-5'-hexyllactone has been synthesised. This rather efficient and flexible 5-step approach was applied for the stereoselective synthesis of 3'-deoxylactone **176**. The stereocenter was introduced selectively via HNL-catalysed hydrocyanation. The subsequent epoxidation and hydrolysis-induced lactonisation proceed with high diastereoselectivity.

6.1 Introduction

Deoxysugars are an important class of carbohydrates that occur widely in nature. They are commonly defined as monosaccharides with one or more hydroxyl group(s) replaced by *e.g.* hydrogen(s). Many biologically active natural products are glycosylated by one or more deoxysugar saccharide chains. These include important antibiotics (erythromycin)[1], antifungals (amphotericin B)[2], antiparasites (avermectins)[3] and anticancer drugs (doxorubicin).[4] 3'-Deoxysugars also appear in nucleosides that since their discovery about sixty years ago cover some interesting compounds. Cordycepin (3'-deoxyadenosine) possesses several pharmacological activities like antitumour,[5] antifungal,[6] or antiviral activity.[7] Other 3'-deoxy nucleosides, AZT and 3TC,[8e] are known as AIDS therapeutics, which inhibit reverse transcriptases involved in HIV-replication.[8] Due to the lack of the 3'-hydroxy group, these nucleosides act as chain terminators by blocking elongation of nascent DNA, leading to inhibition of viral replication.[8c] 3'-Deoxy nucleosides can be interesting as building block for the synthesis of other pharmaceutically important nucleosides as well, *e.g.* regulators of both the immune system and blood pressure via an agonist-antagonist effect on the G-protein coupled P2-receptors.[9] Selective agonists are believed to be useful as hypotensive agents or in the treatment of diabetes, while selective antagonists may be useful as antithrombotic agents. Furthermore, oligonucleotides like, 2',5'-linked nucleic acids can prevent the expression of specific genes. 2', 5'-Linked nucleic acids possess interesting physiological properties,[10] *e.g.* they show a remarkable preference for complexation with complementary RNA.[10a] Finally, the 3'-deoxysugar moiety also appears in UPA's like mureidomycin A, discussed in chapter 1.

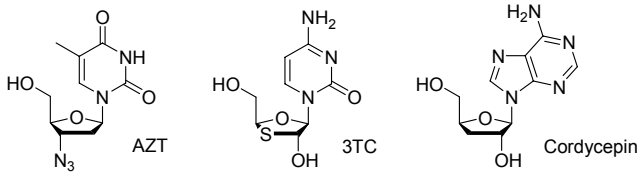
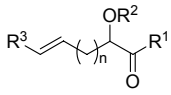


Figure 6.1 Three biologically active 3'-deoxynucleosides

Thus, general and convenient methods for the stereoselective synthesis of rare and/or non-natural carbohydrates and their corresponding nucleosides are of importance and widespread interest. Conventional syntheses of modified carbohydrates and nucleosides are usually based on chiral pool starting materials. The advantage is that the stereochemistry is already set, however natural sugars can only result in targeted molecules with the configurations and standard functionalities provided by nature. This limits the

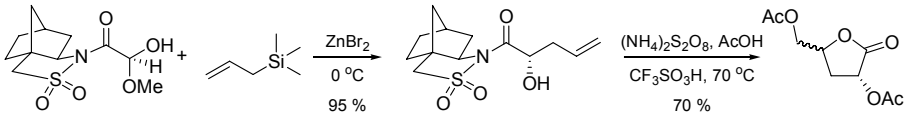
configurational and functional flexibility. Furthermore, these synthetic routes are often lengthy, include difficult separations and are inherently inefficient because the multifunctional and sensitive starting materials limit the synthetic methodology that can be used. Consequently, extensive protection and deprotection reactions are often necessary to synthesise non-natural carbohydrates.



137

Figure 6.2 Starting material for several 3'-deoxylactones

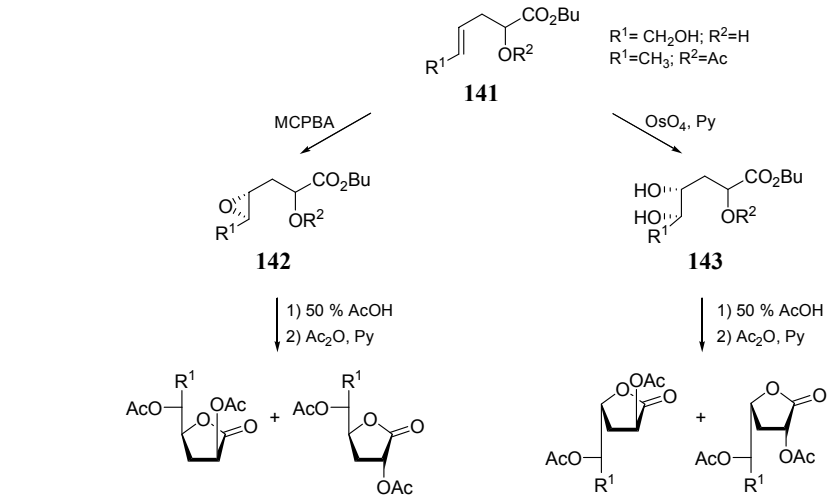
Alternative approaches to access non-natural chiral carbohydrates and their analogues are known, however these syntheses are mostly directed to one specific target molecule. An interesting precursor for the synthesis of 3'-deoxysugars that has been used by others is depicted in Figure 6.2. For example, for the synthesis of 3'-deoxylactone **140** a precursor with a chiral auxiliary attached to **137** was used. The hemiacetal *N*-glyoxyloyl-(*2R*)-bornane-10,2-sultam (**138**)[11] was reacted with allyltrimethylsilane in the presence of a Lewis acid and resulted in compound **139**. This homo-allylalcohol was then acetoxylactonised, which resulted in a 1:1 diastereomeric mixture of lactone **140**. Although this approach is promising, the synthesis of bornane-10,2-sultam **138** is not trivial.[12]



138 **139** (84 % *ee*) **140** (1:1)

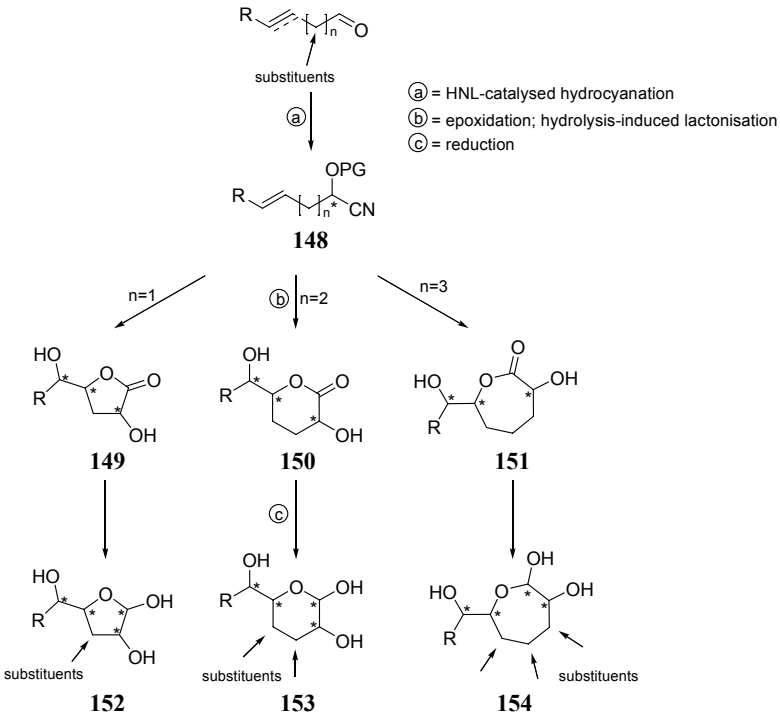
Scheme 6.1 Synthesis of 3'-deoxylactone according to Jurczak.[11,12]

Another approach starts from γ,δ-unsaturated hydroxyl esters **141** as common precursor. The key steps for the asymmetric synthesis of 3'-deoxylactones **144**-**147** are depicted in Scheme 6.2. Epoxidation of the double bond with *m*-chloroperbenzoic acid afforded mixtures containing two stereoisomeric epoxides. Opening of the oxirane ring and lactonisation of the resulting aldonic acids upon treatment with aqueous acetic acid, followed by acetylation, yielded mixtures of arabino (**144**) and ribo (**145**) configurations in 50-60 % yield. The other pair of stereoisomers was obtained by direct cis-hydroxylation of the double bond in **141**, followed by lactonisation and acetylation. This led to a mixture of two isomeric γ-lactones with xylo (**146**) and lyxo (**147**) configuration in 50-80 % yield.[13]



Scheme 6.2 Synthesis of lactones **144**–**147**

We envisioned a *de novo* synthesis of 3'-deoxycarbohydrates starting from simple chemicals using stereoselective enzyme reactions (Scheme 6.3). Unsaturated aldehydes, either commercially available or easily obtained via oxidation from the corresponding alcohols (*e.g.* see chapter 5) can be used as starting materials for the synthesis of 3'-deoxycarbohydrates. Subsequent hydrocyanation catalysed by HNL provides optically pure cyanohydrins **148**. Next, epoxidation and hydrolysis-induced lactonisation (either chemically or enzymatically) results in lactones **149-151**. Finally, reduction of the lactones results in 3'-deoxycarbohydrates **152-154**. Depending on the carbon chain between the unsaturated bond and the cyanohydrin functional group 5-, 6-, or 7- membered lactones can be obtained. In this way, the synthetic routes are straightforward and ensure efficient and stereoselective access to a broad spectrum of differently functionalised 3'-deoxycarbohydrates. As displayed in the former chapter a (*S*)-selective HNL enzyme can be applied for the synthesis of cyanohydrins **148**. When used in combination with chemical cascade reactions optimal stereo- and regiocontrol can be achieved.



Scheme 6.3 *De novo* approach to 3'-deoxycarbohydrates

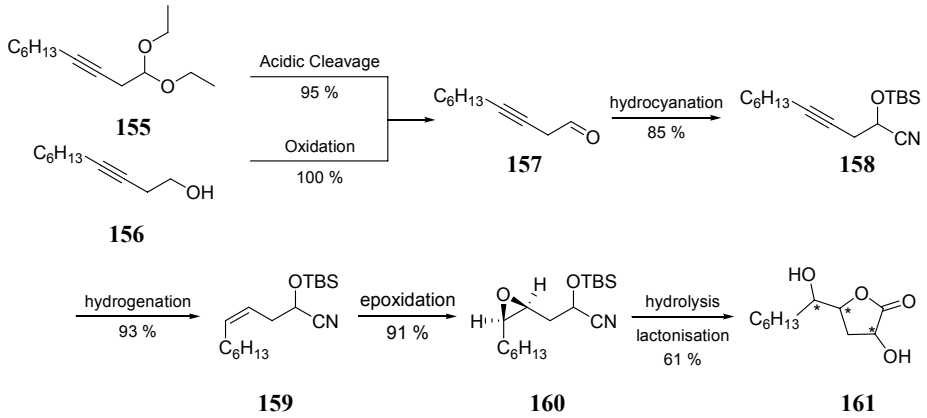
6.2 *Synthesis of 3'-deoxylactone **161***

First we studied the chemical feasibility of the general approach discussed above. For practical reasons aldehyde **157** was chosen as starting molecule. The route depicted in Scheme 6.4 should then lead to the 3'-deoxy lactone **161**. At a later stage chiral building blocks from chapter 5 could be transformed in a similar fashion to the corresponding optically active 3'-deoxyribose moieties.

6.2.1 *Chemical strategy*

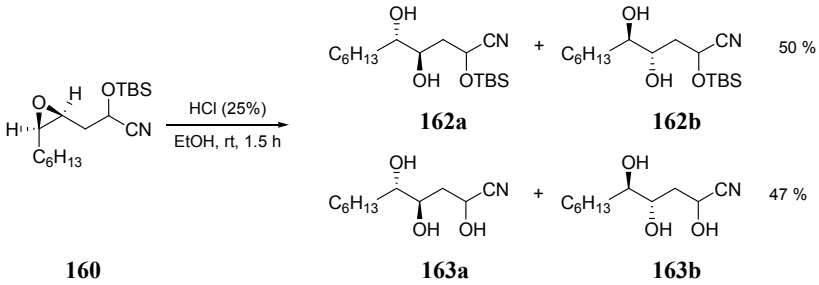
Thus, the racemic synthesis starts from aldehyde **157**. This aldehyde can either be synthesised from a diacetal[14] **155** or an alcohol **156**.[15] Via acidic cleavage of **155** using a 4/1/1 solvent mixture of DCM/TFA/H_2O aldehyde **157** was obtained in 95 % yield.[16] Alternatively, oxidation of the alcohol using 2 equiv. Dess-Martin periodinane (DMP) gave **157** in quantitative yield. Subsequently, aldehyde **157** is transformed into cyanohydrin **158** via Greenlee's method[17] using TBSCN, KCN and 18-crown-6 ether in 85 % yield. After the triple bond of cyanohydrin **158** is hydrogenated with Lindlar catalyst[18] in ethanol in 93 %

yield, the resulting cis-alkene **159** was epoxidised. To achieve this, 5 equiv. oxone® were added to a three-phase system containing alkene **159**, 18-crown-6 ether and $NaHCO_3$.[19] This resulted in a 1:1 mixture of two diastereomers **160** in 91 % yield, which could be separated by column chromatography (CC).



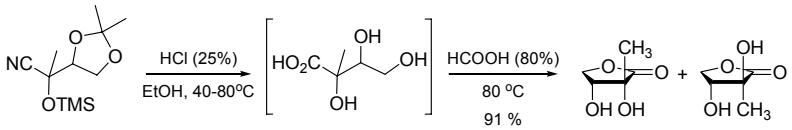
Scheme 6.4 Chemical synthesis of model compound

At this point the key transformation, a hydrolysis-induced lactonisation of epoxides **160** to form the desired lactones **161** was planned. In an initial attempt to achieve this, the epoxides **160** were reacted with 20 equiv. of 25 % HCl at room temperature. According to ^{1}H NMR the diols **162** and **163** were formed in 50 % and 47 % yield, respectively. This indicates that the cyano group survives the acid treatment at room temperature.



Scheme 6.5 Hydrolysis of epoxide **160**

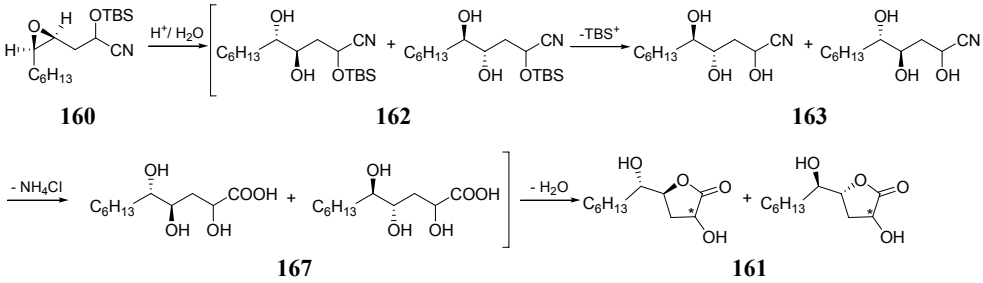
To the best of our knowledge no precedent exists in literature for the hydrolysis-induced lactonisation, starting from a TBS-protected epoxy cyanohydrin like **160**. However, conditions similar to those we tried (see Scheme 6.5) led to efficient hydrolysis-induced lactonisation of TMS-protected cyanohydrin derivative **164** and affords the desired corresponding lactones **166** efficiently (Scheme 6.6).[20]



164 **165** **166** (3:1)

Scheme 6.6 Hydrolysis-induced Lactonisation by Bacher *et al.*[20]

In a further test reaction, **160** was allowed to react with excess HCl (25 %) in refluxing ethanol for 6 hours. After work-up and purification, it was possible to obtain in about 20 % yield a pure product with a characteristic signal at 1779 cm^{-1} in the IR-spectrum. This indicates the formation of stable lactones **161**, which can only be the result of a cascade reaction including 4 succesive steps starting from **160** (Scheme 6.7). After extensive spectroscopic analysis (see section 6.2.2) the structure of the lactones **161** could be confirmed. The order of events are summarised in Scheme 6.7. Acid catalysed epoxide ring opening of **160**, results in threo diols **162** via an anti addition of water. Bulky silyl protective groups are more stable than epoxides, so under highly acidic conditions they will be hydrolysed after the oxirane moiety to give the triols **163**. At this stage, the cyano-group is still intact but under reflux conditions this will be hydrolysed as well, resulting in the carboxylic acids **167**. These will undergo an intramolecular esterification through nucleophilic attack of the C4-hydroxyl group to the electrophilic carbon of the carboxylic group to ultimately give lactones **161**.



160 **162** **163**

167 **161**

Scheme 6.7 Lactonisation of epoxide **160**

The cascade reaction leading to lactones **161** was further optimised. Thus, in the presence of 5 equiv. of acid, the solvent and reaction temperature were varied. In Table 6.1 the results are summarised. In dioxane only small amounts of side products were formed according to GC, and an optimal yield of ~ 84 % was found. An additional advantage of the hydrolysis-induced lactonisation reaction performed in dioxane is that the reaction can be followed visually by the formation of a white pricipitate, which is the NH_4Cl salt. The

precipitation of NH_4Cl may drive the reaction to completion and additional work-up is easier as compared to the reaction in ethanol and *t*-butanol. When the optimised conditions were used for the hydrolysis-induced lactonisation of **160** the lactones **161a-d** were obtained in a ratio of 1:5:6:1 in 61 % isolated yield. The formation of four diastereomeric lactones may be explained by little temperature-induced epimerisation or by non-optimal regioselectivity in the first epoxide-hydrolysis step of the cascade process (9% **161a** and **d**). However, no 6-membered ring lactone formation was observed.

Table 6.1 Optimisation of the cascade reaction using 25 % HCl in different solvents.

Solvent	Yield (%)[a]
EtOH	45
*t*BuOH	10
Dioxane	84

a) Yield was determined by GC.

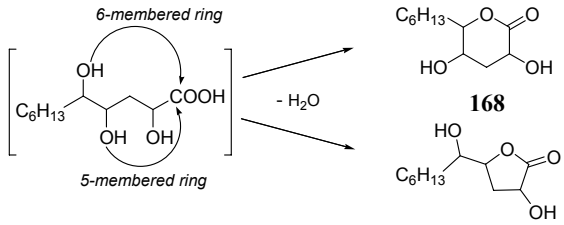
The cis-alkene **159** was employed, which leads to cis-epoxides **160** and in principle to only four of the eight possible stereoisomers of **161**. However, the route is easily modified to yield the corresponding trans-alkenes, *e.g.* by reduction of the triple bond using metal hydride reducing agents instead of hydrogenation. Subsequent analogous transformation should then afford the corresponding trans-epoxides. These then lead to the other four stereoisomers of **161**. In this way all eight possible stereoisomers of lactone **161** are accessible.

*6.2.2 Structure of lactones **161***

Even after extensive crystallisation efforts it was not possible to obtain single crystals suitable for X-ray analysis. This would, of course, have unambiguously proven the structure of the lactones **161**. Our structural elucidation had, consequently, to rely on alternative spectroscopic considerations, which are discussed in the following section.

Kinetics of lactone formation

Many examples of intramolecular reactions leading to ring closure have served to establish a correlation between the rate of a reaction and the ring size of the ring being formed. Intramolecular lactonisation from carboxylic acid intermediate **167** may result in two possible products, a five- or a six-membered ring lactone (Scheme 6.8). Illuminati *et al.* have investigated the lactonisation of ω-bromoalkanoic acids with strong bases in 99 % aqueous DMSO to determine the rate constants for ring formation in order to obtain deeper understanding of the factors involved in ring closure.[21]



Scheme 6.8 Mechanism for 5- and 6-membered lactone formation

The experiments showed first-order rates, which are collected in Table 6.2. Formation of 5-ring lactones are approximately 100 times faster, compared to formation of corresponding 6-ring lactones under these conditions. Although the conditions are not directly comparable to the hydrolysis-induced lactonisation reaction described here, it shows that formation of 5-ring lactones like **161** is fairly easy as compared to the corresponding 6-ring lactones.

Table 6.2 Relative rates for the formation of lactones[21]

Ring size	Relative rate
4	$2.4 \cdot 10^2$
5	$2.9 \cdot 10^4$
6	$2.6 \cdot 10^2$
7	1

IR absorption frequency of lactones

Lactones have been extensively studied on their characteristic frequencies in the infrared spectra. Rasmussen[22] found that a γ-valerolactone (five-membered ring) has a characteristic frequency of 1770 cm^{-1} and Jones[23] found 1780–1778 cm^{-1} for steroid γ-lactones. Generally, saturated five-membered ring lactones show a C=O stretch frequency between 1780–1760 cm^{-1}. For six-membered rings (δ-lactones) and also the open-chain analogues characteristic C=O stretch frequencies are observed between 1750–1735 cm^{-1}. This can be attributed to the lack of strain in the ring.[23] The order of the C=O stretch frequency for lactones is: 4-ring > 5-ring > 6-ring = acyclic (1818, 1775, 1740cm^{-1} respectively). Bartlett and Stiles explained this effect in terms of the hybridisation of the carbon atom of the carbonyl group, rather than strain in the ring.[24] As the ring is contracted, the bonds to this carbon atom have more *p*-character, which confers more *s*-character to the C=O bond. This strengthening of the carbonyl bond will be reflected in a higher force constant and hence in a higher C=O stretch frequency.[25]

The above considerations show that for lactones the IR C=O stretch frequencies are a reliable tool for characterisation. Upon comparison of the C=O stretch frequencies of the IR spectra of lactones **161a-d** with those reported for lactones in general (see above) the data confirm that these indeed are 5-ring lactones.

Table 6.3 IR absorption frequencies of lactone **161**

Lactone	λ (cm^{-1})
161a	1777
161b+d	1773
161c	1757
five-membered ring	1780-1760[23]
six-membered ring	1750-1735[23]

a) **161b**+**d** was measured as a mixture, which could not be separated by CC.

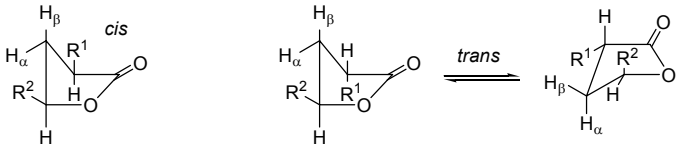
*NMR studies on lactone **161***

Additional evidence for the structure of **161** was collected as well. Thus, the ^{13}C signals of the lactone C=O of **161a-c** were compared with ^{13}C NMR data from literature (Table 6.4). These data also support the proposed 5-ring lactone structure for **161**.

Table 6.4 ^{13}C NMR data of lactones

Lactone	δ (ppm)
161a	176.8
161b+d	177.1
161c	176.2
five-membered ring	178.7[26] and 178.1[27]
six-membered ring	172.4[26] and 171.2[27]

The conformational behaviour of 5-memberered ring lactones is more complex and less well understood than that of 6-membered ring lactones. It is thought that the reason for this is associated with the flexible nature of five-membered rings, which permits rapid pseudorotation involving interconversion of numerous conformations of similar energies. However, in 2,4-disubstituted-γ-butyrolactones, pseudorotation is probably severely impaired by either the presence of substituents on C2 and C4, and/or the resonance demands of the C-CO-O-C group for planarity.[28] Although this is an oversimplification, qualitative conclusions can be drawn. 2,4-Disubstituted-γ-butyrolactones can exist in a conformation in which both substituents are quasi-equatorial (cis-isomer; **169**) or in a conformation where one of the two substituents is quasi-axial. (trans-isomer; **170** and **171**).



169 **170** **171**

Figure 6.3 Possible conformations of 2,4-disubstituted -γ-butyrolactones

Although calculation of specific torsional angles in flexible systems from vicinal constants are a dangerous procedure and is to be avoided, the relative magnitudes characterise the different diastereomers. When the vicinal coupling constants of lactones **161a-d** are compared with other 2,4-disubstituted-γ-butyrolactones,[28] one can conclude that **161a** is similar to **161b** and this corresponds to the trans conformation because the pattern of vicinal couplings is: $2 - 3\beta > 2 - 3\alpha$ and $3\alpha - 4 > 3\beta - 4$. Lactone **161c** is similar to **161d,** being the *cis*-isomers, because: $2 - 3\alpha > 2 - 3\beta$ and $3\alpha - 4 > 3\beta - 4$.

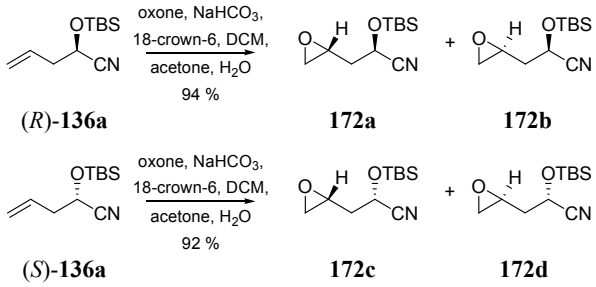
Table 6.5 Coupling constants of all 4 stereoisomers

$^3J_{HH}$	**161a** (Hz)	**161b** (Hz)	**161c** (Hz)	**161d** (Hz)
$2 - 3\alpha$	7.0	7.5	10.6	10.7
$2 - 3\beta$	8.8	9.2	8.7	8.7
$3\alpha - 4$	8.5	9.0	10.5	9.8
$3\beta - 4$	3.6	3.1	5.6	5.6

*6.3 Synthesis of lactones **176***

From the previous section it becomes clear that 5-membered ring lactones can be synthesised in 5 steps from a simple alcohol in 44 % overall yield. The key step in this sequence is the four-reaction hydrolysis-induced lactonisation cascade. The results described in chapter 5 show that optically active cyanohydrins can be obtained from the corresponding alcohols by TEMPO/$PhI(OAc)_2$ mediated oxidation followed (in situ) by a stereoselective HNL-catalysed hydrocyanation. Via this protocol the γ,δ-unsaturated TBS-protected cyanohydrins (*S*)-**136a** can be obtained by employing an (*S*)-selective HNL (see chapter 5; p. 115-118). Further elaboration of this material via the general route described in the previous section would lead to optically active 3'-deoxylactones. After reduction, these yield 3'-deoxyribose derivatives, which are useful as chiral building blocks for the synthesis of mureidomycin analogs (see also scheme 1.4 in chapter 1). Thus, (*R*)-**136a** and (*S*)-**136a**[29] were epoxidised with oxone® resulting in epoxides **172a-d** in 94 or 92 % yield,

respectively. Both diastereomers are formed in about a 1:1 ratio. When (*R*)-**136a** was employed as starting material, the two diastereomers could be separated by CC.



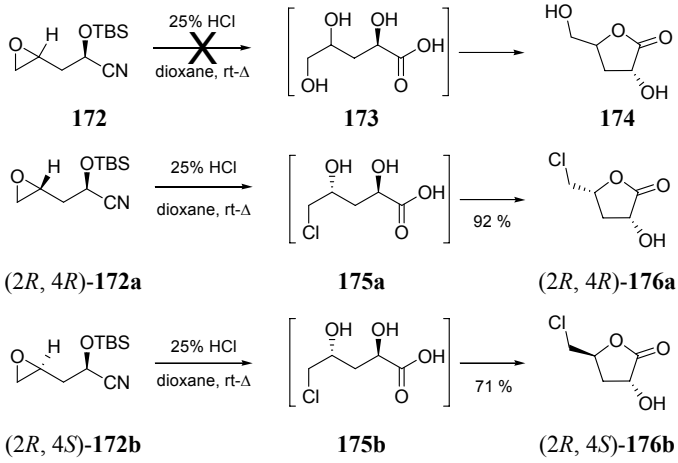
(*R*)-**136a** **172a** **172b**

(*S*)-**136a** **172c** **172d**

Scheme 6.9 Synthesis of epoxides **172**

The subsequent hydrolysis induced lactonisation, was performed following the procedure described for epoxide **160**. Epoxides **172a** and **b** were separately treated in this four-step cascade reaction. However, not the expected lactones (**174**) with a 5'-OH substituent, but the lactones with a 5'-chloro substituent were formed (**176**). This is confirmed with LRMS and HRMS. The formation of this chlorolactone **176**, is the result of attack of the chloride at the primary carbon and not at the secondary carbon of the epoxide, resulting in retention of the stereochemistry. Additional hydrolysis of the TBS-protected cyanohydrin results in intermediates **175a** and **b**, which upon ring-closure result in chlorolactones **176a** and **b**.

Similar 5'-iodolactones have been synthesised before.[30] However, never an enantiopure starting material was used for the lactonisation. Thus always resulting in either a racemic mixture[30a] of lactones or one pair of diastereomers. [30b]



172 **173** **174**

(2*R*, 4*R*)-**172a** **175a** (2*R*, 4*R*)-**176a**

(2*R*, 4*S*)-**172b** **175b** (2*R*, 4*S*)-**176b**

Scheme 6.10 Synthesis of lactones

6.4 Conclusions

The synthesis of 3'-deoxylactones can be accomplished starting with simple chemicals, like β,γ-unsaturated aldehydes. 5'-Hexyl 3'-deoxylactone **161** can be synthesised in 5 steps from the commercially available 3-decyn-1-ol in 44 % overall yield. This strategy is also used for the steroeselective synthesis of 3'-deoxylactones **176**. With this we developed an interesting straightforward and stereoselective synthesis of 3'-deoxylactones, which can be elaborated to 3'-deoxycarbohydrates as displayed in Scheme 6.3.

6.5 Acknowledgements

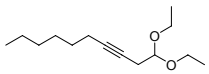
We thank Dr. Marek Smoluch for measuring the HRMS samples and Rian van den Nieuwendijk from Leiden University for the generous gift of (*R*)-**136a**.

6.6 Experimental section and physical data

6.6.1 General part

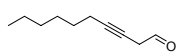
^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 (250.13 and 62.90, respectively) or a Bruker MSL 400 (400.13 and 100.61 MHz respectively) spectrometer; chemical shifts (δ) are given in ppm, internally referenced to residual solvent resonances (^{1}H: δ 7.29 ppm, ^{13}C: δ 77.0 ppm). Column chromatography was performed with Baker 7024-02 silica gel (40 μ, 60 Å) with solvents petroleum ether (PE, boiling range 40-60 °C), ethyl acetate (EA) or diethyl ether (DEE). Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC were visualised by UV-detection and/or coloured with MOP solution (14g phosphomolybdic acid in 125 ml EtOH), anisaldehyde solution or CERMOP solution (5g phosphomolybdic acid, 2g $Ce(SO_4)_2$ and 16 ml sulphuric acid in 185 ml of demiwater) followed by heating to 150˚C. High-resolution mass spectra (HRMS, EI) were recorded on a Finnigan Mat 900 spectrometer at 70 eV. IR spectra are recorded on a Mattson-6030 Galaxy spectrophotometer and are reported in cm^{-1}. Optical rotation was determined with an AA-10 automatic polarimeter from Optical Activity. Gas Chromatography was performed on INTERSMAT IGC 121 (3% CP-SIL-8, 3m ¼ CHROM-W-HP 100-120 mesh) equipped with a FID. Injector and Detector temperature was 200˚C and flow was 30 ml/min of H_2. Integration was performed with a LDC/Milton Roy CI-10B integrator. Temperature programme is as follows: [T_{start} (˚C) ─ $t_{constant}$ (min.) ─G (˚C/min.) ─ T_{end} (˚C)]. Diethyl ether was distilled from potassium and sodium. THF was distilled from sodium/benzophenone. Dichloromethane was distilled from P_2O_5. Methanol was dried over molecular sieves. Other commercially available chemicals were used as purchased. When necessary the reactions were carried out under dry nitrogen or argon.

6.6.2 Synthetic procedures and Physical Data



1,1-diethoxy-3-yne (155)

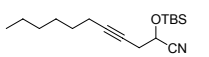
To a vigorously stirred solution of 1-octyne (7.00 mL, 83.5 mmol) in THF (150 mL) was slowly (approx. 1.5 h) added a 1.6 M nBuLi in hexanes solution (43.75 mL, 70.0 mmol) under nitrogen at -65˚C. Subsequent a catalytic amount of HMPA (2.00 mL) and bromoacetaldehyde diethyl acetal (7.65 mL, 50.9 mmol) were slowly added. After the reaction mixture was warmed to room temperature, it was refluxed for three more days, until the reaction was finished (the reaction was followed by GC and TLC). The reaction mixture was washed with a saturated NH_4Cl solution (3×150 mL) and water (3×100 mL). The organic layer was dried over $MgSO_4$, purified with norit, filtered over a layer of high-flow and concentrated under reduced pressure to give a yellow oil. The product was purified by CC (PE/EA 40:1) and obtained as a yellow oil. Yield: 11.1 g, 48.9 mmol, 96 %; Rf (PE/EA 40:1) = 0.30; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=0.82 (t, *J*=6.9 Hz, 3H; C*H*$_3$CH$_2$), 1.15 (t, *J*=7.1 Hz, 6H; C*H*$_3$CH$_2$O), 1.21-1.41 (m, 8H; CH$_3$(C*H*$_2$)$_4$), 2.01-2.09 (m, 2H; C*H*$_2$C), 2.44-2.49 (m, 2H; C*H*$_2$CH), 3.45-3.73 (m, 4H; OC*H*$_2$), 4.54 (t, *J*=5.7 Hz, 1H; C*H*CH$_2$); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=13.9, 15.1 (2C), 18.6, 22.4, 24.9, 28.4, 28.7, 31.2, 61.5 (2C), 74.9, 81.8, 101.1; IR (NaCl): $\tilde{\nu}$= 2931 (m), 2860 (m), 1456 (m), 1373 (m), 1344 (m), 1119 (s), 1065 (s), 1022 (s); HRMS (EI): m/z=227.2007 $[M]^+$, calc. for $C_{14}H_{27}O_2$=227.2011; GC programme [90-3-20-200] t_r=10.4 min.



3-decynal (157)

A) From 1,1-diethoxy-3-decyne **155**: 1,1-diethoxy-3-decyne **155** (2.26 g, 10.0 mmol) was slowly added to a vigorously stirred two-phase system of DCM/TFA/H_2O (100/25/25 mL) at room temperature. To protect the reaction mixture from daylight and in this way prevent side reactions, the flask was covered with a sheet of aluminium. After the reaction ran to completion (5.5 h, according to GC), the excess of TFA was carefully neutralised with a saturated $NaHCO_3$ solution. The organic layer was separated, dried over $MgSO_4$, purified with norit and filtered over a layer of high-flow to give a yellow solution of 3-decynal **157** in dichloromethane. Yield: ~ 95% (according to GC). Due to the sensitive nature of **157**, the organic layer was not concentrated or purified by CC.

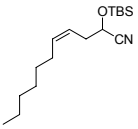
B) From 3-decynol **156** with DMP: 3-Decynol **156** (1.71 mL, 1.4 mmol) was added under nitrogen to a solution of DMP (1.19 g, 2.8 mmol) in DCM (6 mL) at room temperature. The reaction was stirred for 45 min. until full conversion was reached (according to ^{1}H NMR). 3-Decynal **157** was not purified because of its sensitivity. ^{1}H NMR (250. 13 MHz): δ= 0.92 (t, *J*=6.7 Hz, 3H; C*H*$_3$(CH$_2$)$_4$), 1.31–1.55 (m, 8H; CH$_3$(C*H*$_2$)$_4$), 2.20–2.24 (m, 2H; C*H*$_2$C), 3.25 (d, *J*=1.9 Hz, 2H; C*H*$_2$CHO), 9.63 (t, *J*=1.9 Hz, 1H; C*H*O); GC programme [180˚C iso] t_r =1.4 min.



2-(tert-butyldimethylsilyloxy)undec-4-ynenitrile (158)

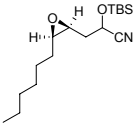
To a solution of TBSCN (0.56 g, 4.0 mmol) KCN (0.02 g, 0.3 mmol), 18-crown-6 (0.13 g, 0.5 mmol) in DCM (10 mL) was slowly added a solution of 3-decynal **157** (2.0 mmol) in DCM at room temperature under nitrogen. After addition was complete, the reaction mixture was stirred for 24 h at room temperature. The reaction mixture was washed with a saturated NH_4Cl solution. The organic layer was separated, dried over $MgSO_4$, purified with norit, filtered over a layer of high-flow and concentrated to give a yellow oil. Purification by CC (PE/EA 40:1) gave a yellow oil of **158**. Yield: 0.52 g, 1.75 mmol, 87 %; Rf (PE/EA 40:1) = 0.35; ^{1}H NMR (250.13 MHz,

$CDCl_3$): δ=0.10 (s, 3H; SiC*H*$_3$), 0.21 (s, 3H; SiC*H*$_3$), 0.86–0.95 (m, 12H; SiC(C*H*$_3$)$_3$, C*H*$_3$(CH$_2$)$_5$), 1.15–1.56 (m, 8H; (C*H*$_2$)$_4$CH$_3$), 2.16–2.18 (m, 2H; (CH$_2$)$_4$C*H*$_2$), 2.65–2.67 (m, 2H; C*H*$_2$CHCN), 4.52 (t, *J*=7.1 Hz, 1H; C*H*CN); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ= -5.5, -5.4, 13.9, 18.5 (2C), 22.4, 25.3 (3C), 27.4, 28.4, 28.5, 31.2, 61.7, 72.8, 84.4, 119.4; IR (NaCl): $\tilde{\nu}$= 2932 (s), 2859 (s), 2235 (w), 1472 (w), 1256 (w), 1117 (s); GC programme [100-2-20-200] t_r =13.0 min.



(Z)-2-(tertbutyldimethylsilyloxy)undec-4-enenitrile (159)

A suspension of Lindlar's catalyst (0.15 g) in EtOH (5 mL) was degassed and subsequently flushed with nitrogen (three times). This was repeated with a balloon of H_2-gas. Subsequently **158** (1.11 g, 3.8 mmol) was added dropwise. After 30 min the reaction was completed (according to TLC and GC). Thereafter, the solution was dried over $MgSO_4$, filtered over a layer of high-flow and concentrated. Purification by CC (PE/DEE 20:1) resulted in a yellow oil of **159**. Yield: 1.04 g; 3.5 mmol; 93%; Rf (PE/DEE 20:1) = 0.39; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=0.11 (s, 3H; C*H*$_3$Si), 0.16 (s, 3H; C*H*$_3$Si), 0.82–0.90 (m, 12H; SiC(C*H*$_3$)$_3$ and C*H*$_3$CH$_2$), 1.25 (br s, 8H; (C*H*$_2$)$_4$CH$_3$), 2.01-2.05 (m, 2H; C*H*$_2$C), 2.48–2.55 (m, 2H; C*H*$_2$CH), 4.36 (t, *J*=6.7 Hz, 1H; C*H*CN), 5.29-5.67 (m, 2H; C*HCH*); ^{13}C NMR (50.32 MHz, $CDCl_3$): δ=-5.5, -5.4, 13.9, 17.9, 22.5, 25.3, 27.4 (3C), 28.8, 29.3, 31.6, 34.2, 61.9, 119.4, 121.2, 135.0; HRMS (EI): m/z=296.2418 $[MH]^+$, calc. for $C_{17}H_{34}NOSi$=296.2410; MS (EI) m/z (%): 281 (45), 221 (10), 147 (20), 115 (30), 83 (100); GC programme [180°C iso], t_r =11.0 min.



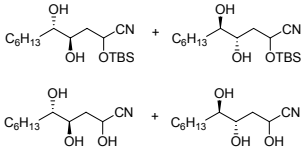
***cis*-1-(TBDMSO)-1-cyano-3,4-epoxydecane (160)**

To a solution of cis-alkene **159** (1.49 g, 5.1 mmol) in DCM (145 mL), acetone (145 mL), water (45 mL), $NaHCO_3$ (12.6 g, 150.0 mmol) and 18-crown-6 ether (0.11 g, 0.4 mmol) were successively added. The resulting three-phase system was cooled to 0°C and oxone® (23.3 g, 37.9 mmol) dissolved in water (100 mL) was added dropwise (1drop/sec.). After overnight stirring, the reaction was complete (according to GC and TLC) and a $Na_2S_2O_5$-solution (5%, 80 mL), a saturated $NaHCO_3$ solution (80 mL) were added. The reaction mixture was salted with NaCl. The organic layer was separated and the aqueous layer was extracted with DCM (2×100 mL) and DEE (2×100 mL). The combined organic layer were dried ($MgSO_4$), purified with norit, filtered over high-flow and concentrated to give a colourless oil. Purification by CC (PE/DEE 15:1) gave a 1:1 mixture of diastereomers **160a** and **b**. Yield: 1.44 g, 4.61 mmol, 91 %;

160a Rf (PE/DEE 9:1) = 0.45; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.16 (s, 3H; C*H*$_3$Si), 0.21 (s, 3H; C*H*$_3$Si), 0.87 (s, 3H; C*H*$_3$CH$_2$), 0.92 (s, 9H; (C*H*$_3$)$_3$C), 1.25-1.59 (m, 10H; (C*H*$_2$)$_5$CH$_3$), 1.92 (ddd, *J*=5.8 Hz, *J*=7.5 Hz, *J*=14.2 Hz, 1H; C*H*CHCN), 2.12 (ddd, *J*=4.5 Hz, *J*=7.8 Hz, *J*=14.2 Hz, 1H; C*H*CHCN), 2.98 (dt, *J*=4.3 Hz, *J*=6.6 Hz, 1H; CHO (epoxide)), 3.11 (dt, *J*=4.5 Hz, *J*=7.5 Hz, 1H; CHO (epoxide)), 4.61 (dd, *J*=5.8 Hz, *J*=7.8 Hz, 1H; C*H*CN); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= -5.4, -5.2, 14.0, 18.0, 22.5, 25.5 (3C), 26.5, 28.0, 29.1, 31.7, 35.3, 52.4, 56.6, 60.4, 119.4;

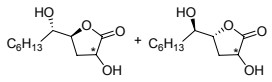
160b Rf (PE/DEE 9:1) = 0.53); ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.18 (s, 3H; C*H*$_3$Si), 0.23 (s, 3H; C*H*$_3$Si), 0.89 (s, 3H; C*H*$_3$CH$_2$), 0.93 (s, 9H; (C*H*$_3$)$_3$C), 1.25-1.59 (m, 10H; (C*H*$_2$)$_5$CH$_3$), 1.81 (ddd, *J*=3.5 Hz, *J*=8.1 Hz, *J*=14.1 Hz, 1H; C*H*CHCN), 2.12 (ddd, *J*=4.0 Hz, *J*=9.5 Hz, *J*=14.1 Hz, 1H; C*H*CHCN), 2.98 (dt, *J*=4.3 Hz, *J*=7.5 Hz, 1H; CHO (epoxide)), 3.11 (dt, *J*=4.0 Hz, *J*=8.1 Hz, 1H; CHO (epoxide)), 4.61 (dd, *J*=3.5 Hz, *J*=9.5 Hz, 1H; C*H*CN); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= -5.5, -5.3,14.0, 18.1, 22.5, 25.5 (3C), 26.4, 28.0, 29.1, 31.7, 35.1, 52.4, 57.6, 59.6, 119.7; IR (NaCl): $\tilde{\nu}$= 2956 (m), 2931 (m), 2860 (m), 1471 (s), 1255 (s), 1119 (s), 839 (s), 783 (s); HRMS (EI):

m/z=312.2312 $[MH]^+$, calc. for $C_{17}H_{34}NO_2Si$=312.2359; GC programme [200˚C iso], t_r **160a** = 7.0 min. and t_r **160b** = 7.4 min.



Diols 162 and **Triols 163**

To a solution of *cis*-1-(TBDMSO)-1-cyano-3,4-epoxydecane **160** (0.16 g, 0.50 mmol) in EtOH (1mL) was added dropwise HCl (1 mL 25 %) at room temperature. After 90 min. the reaction was stopped via neutralisation with NH_3 (25% in water) and extracted with PE (3×10 mL). The combined organic layer was dried over $MgSO_4$, filtered over high-flow and concentrated to give a yellow oil. Purification by CC (PE/DEE 9:1) gave a 1:1 mixture of diols **162** and triols **163**. Yield: (**162**: 83 mg; 0.25 mmol; 50% and **163**: 51 mg; 0.24 mmol; 47%). ^{1}H NMR and ^{13}C NMR spectra revealed that the products were the diols and triols, however the triols contained too many isomers to be interpreted. **162**: ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.17 (s, 3H; CH_3Si), 0.18 (s, 3H; CH_3Si), 0.21 (s, 3H; CH_3Si), 0.22 (s, 3H; CH_3Si), 0.87-0.94 (m, 6H; CH_3CH$_2$), 0.93 (s, 9H; C(CH_3)$_3$), 0.94 (s, 9H; C(CH_3)$_3$), 1.28-1.60 (m, 16H; CH$_3$(CH_2)$_4$), 1.75-1.90 (m, 4H; CH_2(CH$_2$)$_4$), 2.01-2.13 (m, 4H; C*H*CHCN), 2.16 (d, *J*=4.1 Hz, 1H, OH), 2.28 (d, *J*=4.1 Hz, 1H, OH), 3.87-3.95 (m, 4H, C*H*OH), 4.68-4.75 (m, 2H; C*H*CN); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= -5.6, -5.4, -5.3, -5.2, 14.0 (2C), 18.0 (2C), 22.5 (2C), 25.5 (6C), 26.4 (2C), 28.7 (2C), 31.6 (2C), 34.3, 34.7, 40.7, 41.1, 58.7, 60.1, 68.1, 68.4, 69.2, 70.7, 119.6, 120.0; HRMS (EI): m/z=330.2476 $[MH]^+$, calc. for $C_{17}H_{36}NO_3Si$=330.2465.



Lactones 161a–d

To a solution of epoxide **160** (0.33 g, 1.1 mmol) in dioxane (4 mL) was added dropwise 25 % HCl (0.6 mL, 6 mmol) at room temperature. After 15 min, the mixture was warmed to 50˚C, and kept at this temperature for 30 min., subsequently the reaction mixture is refluxed for 2 h. After separating the organic layer, the aqueous layer was extracted with PE/EE (1:1) (3×5 mL). The combined organic layer was dried over $MgSO_4$, filtered over high-flow and concentrated to give a viscous, brown oil. Purification by CC (PE/DEE 1:1) gave a mixture of **161a**, **161b**+**d** and **161c** in 60 % total isolated yield.[31]

161a: Yield: 11 mg; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ= 0.91 (t, *J*=6.7 Hz, 3H; CH_3CH$_2$), 1.25-1.50 (m, 7H; CH_2), 1.55–1.89 (m, 3H, CH_2), 2.33 (ddd, *J*=7.0 Hz, *J*=8.5 Hz, *J*=13.9 Hz, 1H; CH_2 (ring)), 2.61 (ddd, *J*=3.7 Hz, *J*=8.8 Hz, *J*=13.9 Hz, 1H; CH_2 (ring)), 3.02 (br s, 1H; OH), 4.02–4.08 (m, 1H; C*H*OH), 4.63 (dd, *J*=7.0 Hz, *J*=8.8 Hz, 1H; C*H*OH (ring)), 4.70 (ddd, *J*=3.7 Hz, *J*=5.0 Hz, *J*=8.5 Hz, 1H; C*H* (ring)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= 14.0, 22.5, 26.0, 28.6, 31.5, 31.7, 34.0, 63.7, 67.2, 79.7, 176.8; IR (NaCl): $\tilde{\nu}$= 3407 (br s), 2928 (s), 1777 (s), 1458 (m), 1192 (m), 1117 (m).

161b: Yield: 50 mg;[32] ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.90 (t, *J*=6.6 Hz, 3H; CH_3CH$_2$), 1.25-1.60 (m, 7H; CH_2), 1.52–1.62 (m, 1H, CH_2), 1.75–1.92 (m, 2H, CH_2CHOH), 2.39 (ddd, *J*=7.5 Hz, *J*=9.0 Hz, *J*=13.8 Hz, 1H; CH_2 (ring)), 2.59 (ddd, *J*=3.1 Hz, *J*=9.2 Hz, *J*=13.7 Hz, 1H; CH_2 (ring)), 2.86 (br s, 1H; OH), 3.95 (ddd, *J*=2.4 Hz, *J*=5.4 Hz, *J*=9.0 Hz, 1H; C*H*OH), 4.73 (dd, *J*=7.5 Hz, *J*=9.2 Hz, 1H; C*H*OH (ring)), 4.81 (ddd, *J*=2.4 Hz, *J*=3.1 Hz, *J*=9.0 Hz, 1H; C*H* (ring)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= 13.9, 22.4, 26.5, 28.4, 31.4, 33.4, 34.3, 64.7, 66.7, 78.9, 177.1; IR (NaCl): $\tilde{\nu}$= 3403 (br s), 2928 (s), 1773 (s), 1194 (m), 1119 (m).

161c: Yield: 58 mg; ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=0.90 (t, *J*=6.3 Hz, 3H; CH_3CH$_2$), 1.27–1.40 (m, 6H; (CH_2)$_3$CH$_3$), 1.40–1.50 (m, 1H; CH_2CH$_2$CHOH), 1.52–1.67 (m, 1H; CH_2CH$_2$CHOH),1.80–

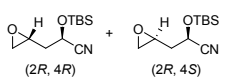
1.86 (m, 2H; CH_2CHOH), 2.23 (ddd, *J*=10.5 Hz, *J*=10.5 Hz, *J*=12.7 Hz, 1H, CH_2 (ring)), 2.69 (ddd, *J*=5.6 Hz, *J*=8.7 Hz, *J*=12.7, 1H, CH_2 (ring)), 3.06 (br s, 2H; OH), 3.97 (dt, *J*=4.5 Hz, *J*=9.1 Hz, 1H; C*H*OH), 4.54 (ddd, *J*=4.5 Hz, *J*=5.6 Hz, *J*=10.1, 1H; C*H* (ring)), 4.58 (dd, *J*=8.7 Hz, *J*=10.5 Hz, 1H; C*H*OH (ring)); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ= 14.0, 22.5, 26.3, 28.6, 31.5, 33.4, 33.5, 62.2, 68.2, 77.9, 176.2; IR (NaCl): $\tilde{\nu}$=3447 (br s), 2928 (s), 1757 (s), 1213 (m), 1144 (m), 1003 (m).

161d: Yield: 12 mg.[32]

Products **161a-d**: MS (EI) m/z (%): 235 (100), 219 (60), 203 (30); GC programme [100-0-4-200], t_r **161a** = 25.33 min.; t_r **161b** = 25.97 min.; t_r **161c** = 25.60 min.; t_r **161d** = 25.57 min.

2-(tbutyldimethylsilyloxy)pent-4-enenitrile (**136a**)

A solution of TBSCN (0.44 g, 3.10 mmol) KCN (0.015 g, 0.23 mmol), 18-crown-6 (0.10 g, 0.37 mmol) in DCM (2 mL) was slowly added to a solution of 3-butenal (**131a**) (1.0 mmol) in DCM and DEE at room temperature under nitrogen atmosphere. After addition was complete, the reaction mixture was stirred for 71 h at room temperature. The reaction mixture was washed with a saturated NH_4Cl solution (5×20 mL), and the resulting aqueous layer was extracted with DCM (4×20 mL). The combined organic layer was dried over $MgSO_4$, purified with norit and concentrated under reduced pressure. Purification by CC (PE/DEE = 94:6) gave a light yellow liquid. According to ^{1}H NMR, the remaining product consisted of a mixture of TBSCN, TBSOAc and 2-(*t*-butyldimethyl-silanyloxy)-pent-4-enenitrile in the molar ratio 0.12:0.62:1.0 (estimated 63 % w/w 2-(*t*-butyldimethylsilanyloxy)-pent-4-enenitrile; 0.14 g; 0.66 mmol; 65 %). ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.17 (s, 3H; CH_3Si), δ 0.22 (s, 3H; CH_3Si), δ 0.94 (s, 9H; SiC(CH_3)$_3$), 2.53–2.59 (m, 2H; CH_2CH), 4.47 (t, *J*= 6.5 Hz, 1H; C*H*CN), 5.23–5.30 (m, 2H; =CH_2), 5.84 (ddt, *J*=17.4 Hz, *J*=9.8 Hz, *J*=7.0 Hz, 1H; C*H*=CH$_2$); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ= -4.9, -4.75, 18.5, 25.9 (3C), 41.1, 62.3, 120.0, 120.5, 132.0; IR (NaCl): $\tilde{\nu}$= 2956 (s), 2860 (s), 1652 (m), 1558 (m), 1259 w), 1114 (m); HRMS (EI): *m/z* = 211.1392 (M^+), calc. for $C_{11}H_{21}NOSi$ = 211.1392.



Epoxides 172a-b

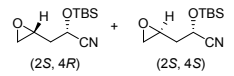
To a solution of (*R*-**136a**) (99 % *ee*; 0.19 g; 0.9 mmol) in DCM (13 mL), acetone (13 mL), water (3.8 mL), $NaHCO_3$ (2.20 g, 26 mmol) and 18-crown-6 ether (0.019 g, 0.07 mmol) were successively added. The resulting three-phase system was cooled to 0°C and oxone® (3.15 g, 5.1 mmol) dissolved in water (17 mL) was added dropwise over 30 min. After stirring overnight, a second portion of DCM (13 mL), acetone (13 mL), H_2O (3.8 mL) and $NaHCO_3$ (2.20 g, 26 mmol) were added. The system was cooled again to 0 °C and oxone® (3.15 g, 5.1 mmol) in H_2O (17 mL) was added over 30 min. After stirring overnight, a third identical portion of DCM, acetone, H_2O, $NaHCO_3$ and oxone was added. To the three-phase system a saturated $NaHCO_3$ solution (20 mL) and 5 % $Na_2S_2O_5$ solution (20 mL) was added dropwise, followed by extraction with DEE (20 mL), DCM (2×20 mL) and DEE (2×20 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by CC (gradient starting from DEE/pentane=1:14 ending with Et_2O/pentane=1:2) gave two different diastereomers. Total yield of the diastereomers is 94 % (194 mg; 0.86 mmol).

Product (2*R*,4*R*)-**172a**: $[\alpha]^{30}_D$ = + 55.0 ° (c =1.0; $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.21 (s, 3H; CH_3Si), 0.26 (s, 3H; CH_3Si), 0.96 (s, 9H; SiC(CH_3)$_3$), 1.85 (ddd, *J*=14.1 Hz, *J*=7.4 Hz, *J*=3.7 Hz, 1H; CH_2), 2.21 (ddd, *J*=14.1 Hz, *J*=8.9 Hz, *J*=4.2 Hz; 1H; CH_2), 2.63 (dd, *J*=4.9 Hz, *J*=2.6 Hz, 1H; CH_2O), 2.90 (dd, *J*=4.9 Hz, *J*=3.9 Hz, 1H; CH_2O), 3.13 (dddd, *J*=7.3 Hz, *J*=4.2 Hz, *J*=3.9 Hz, *J*=2.6

Hz, 1H; C*H*O), 4.67 (dd, *J*=8.9 Hz, *J*=3.7 Hz, 1H; C*H*CN); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ= -5.1, -4.9, 18.5, 25.9 (3C), 40.0, 48.0, 48.3, 59.8, 119.9.

Product (2*R*,4*S*)-**172b**: $[\alpha]^{34}_D$= + 35.9 ^{o} (c = 1.0; $CHCl_3$);[33] ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.19 (s, 3H; CH_3Si), 0.24 (s, 3H; CH_3Si), 0.95 (s, 9H; SiC(CH_3)$_3$), 1.96–2.15 (m, 2H; CH_2), 2.60 (dd, *J*=4.9 Hz, *J*=2.6 Hz, 1H; CH_2O), 2.87 (dd, *J*=4.9 Hz, *J*=4.0 Hz, 1H; CH_2O), 3.11–3.18 (m, 1H; C*H*O), 4.65 (dd, *J*=7.0 Hz, *J*=5.9 Hz, 1H; C*H*CN); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ= -5.0, -4.8, 18.4, 25.9 (3C), 40.0, 47.1, 48.3, 60.3, 119.7.

Products (2*R*,4*R*)-**172a** and (2*R*,4*S*)-**172b**: IR (KBr): $\tilde{\nu}$= 3057 (m), 2959 (m), 2932 (m), 2859 (m), 1472 (s), 1258 (s), 1111 (s), 1015 (s), 899 (s); MS (EI) m/z (%): 199 (6), 170 (18), 143 (29), 115 (100).



Epoxides 172c-d

To a solution of (*S*-**136a**) (93 % *ee*; 0.85 g; 4.0 mmol) in DCM (60 mL), acetone (60 mL), water (16.8 mL), $NaHCO_3$ (10.0 g, 119 mmol) and 18-crown-6-ether (0.09 g, 0.34 mmol) were successively added. The resulting three-phase system was cooled to 0˚C and oxone® (14.0 g, 22.8 mmol) dissolved in water (60 mL) was added dropwise over 2 h. After stirring overnight, a second portion of DCM (60 mL), acetone (60 mL), H_2O (16.8 mL) and $NaHCO_3$ (10.0 g, 119 mmol) were added. The system was cooled again to 0 ^{o}C and oxone® (14.0 g, 22.8 mmol) in H_2O (60 mL) was added over 2 h. After stirring overnight, a third identical portion of DCM, acetone, H_2O, $NaHCO_3$ and oxone was added. To the three-phase system a saturated $NaHCO_3$ solution (90 mL) and 5 % $Na_2S_2O_5$ solution (90 mL) was added dropwise, followed by extraction with DEE (90 mL), DCM (2×90 mL) and DEE (2×90 mL). The combined organic layer was dried over $MgSO_4$ and concentrated under reduced pressure. Purification by CC (gradient starting from DEE/pentane=1:14 ending with DEE/pentane=1:2) gave two different diastereomers in a 1:1 ratio.

Product (2*S*,4*R*)-**172c** and (2*S*, 4*S*)-**172d**: Yield: 92 % (0.85 g; 3.68 mmol); $[\alpha]^{23}_D$ = – 40.0 ^{o}, (c =1.0; $CHCl_3$);[34] ^{1}H NMR (250 MHz, $CDCl_3$): δ=0.06 (s, 3H; CH_3Si), 0.08 (s, 3H; CH_3Si), 0.11 (s, 3H; CH_3Si), 0.13 (s, 3H; CH_3Si), 0.82 (s, 9H; SiC(CH_3)$_3$), 0.83 (s, 9H; SiC(CH_3)$_3$), 1.86–2.10 (m, 4H, CH_2), 2.46–2.53 (m, 2H; CH_2O), 2.73–2.80 (m, 2H; CH_2O), 3.02–3.03 (m, 2H; C*H*O), 4.49–4.57 (m, 2H; C*H*CN); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=-5.6, -5.5, -5.4, -5.3, 17.9 (2C), 25.4 (6C), 39.5 (2C), 46.5, 47.4, 47.7, 47.8, 59.3, 59.8, 119.2, 119.4; IR (NaCl): $\tilde{\nu}$= 2958 (m), 2933 (m), 2860 (m), 1473 (s), 1257 (s), 1124 (s), 839 (s); Products (2*S*,4*R*)-**172c** and (2*S*,4*S*)-**172d**: IR (KBr): $\tilde{\nu}$= 3056 (m), 2959 (m), 2931 (m), 2859 (m), 1472 (s), 1258 (s), 1111 (s), 1015 (s), 899 (s); MS (EI) m/z (%): 170 (18), 143 (29), 115 (100), 75 (25).

(2*R*, 4*R*)-Lactone 176a

To a solution of epoxide (2*R*,4*R*)-**172a** (0.049 g, 0.22 mmol) in dioxane (0.9 mL) was added dropwise 25 % HCl (0.12 mL, 1.2 mmol) at room temperature. After 15 min, the mixture was warmed to 50˚C, and kept at this temperature for 30 min., subsequently the reaction mixture is refluxed for 2.5 h. After separating the organic layer, the aqueous layer was extracted with pentane/DEE (1:1) (3 x 1 mL). The combined organic layer was dried over Na_2SO_4 and concentrated to give a viscous yellow oil of product (2*R*,4*R*)-**176a** (0.030 g; 0.20 mmol; 92%). $[\alpha]^{31}_D$ = + 16.9 ^{o} (c =0.83; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.14 (ddd, *J*=12.8 Hz, *J*=10.2 Hz, *J*=9.9 Hz, 1H; CH_2CHOH), 2.76 (ddd, *J*=12.8 Hz, *J*=8.6 Hz, *J*=5.6 Hz, 1H; CH_2CHOH), 3.72 (dd, *J*=11.8 Hz, *J*=5.4 Hz, 1H; CH_2Cl), 3.73 (dd, *J*=11.8 Hz, *J*=5.3 Hz, 1H, CH_2Cl), 4.58 (dd, *J*=10.2 Hz, *J*=8.6 Hz, 1H;

C*H*OH), 4.62 (ddt, *J*=9.9 Hz, *J*=5.6 Hz, *J*=5.4 Hz, 1H; C*H*CH_2Cl); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=34.7, 45.3, 68.6, 75.7, 176.7; IR (KBr): $\tilde{\nu}$= 3370 (m), 2959 (m), 1775 (s), 1192 (s), 1128 (s); MS (EI) m/z (%): 150/152 (1) [M^+], 149/151 (8), 131/133 (10), 101 (27), 73 (100), 57 (82); HRMS (EI): m/z= 150.0063; calc. for $C_5H_7ClO_3$=150.0084.

(2*R*, 4*S*)-Lactone 176b

Prepared by the same procedure as described for (2*R*,4*R*)-**176a**. In this reaction (2*R*,4*S*)-**172b** (0.053 g, 0.23 mmol) was used. After work up a viscous yellow oil of product (2*R*,4*S*)-**176b** was obtained (0.025 mg; 0.17 mmol; 71 %). ; $[\alpha]^{31}_D$ = + 41.8 ^{o} (c =1.1; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=2.41 (ddd, *J*=13.7 Hz, *J*=8.6 Hz, *J*=7.6 Hz, 1H; CH_2CHOH), 2.57 (ddd, *J*=13.7 Hz, *J*=8.8 Hz, *J*=3.3 Hz, 1H; CH_2CHOH), 3.66 (dd, *J*=12.1 Hz, *J*=3.5 Hz, 1H; CH_2Cl), 3.77 (dd, *J*=12.1 Hz, *J*=4.5 Hz, 1H; CH_2Cl), 4.67 (dd, *J*=8.8 Hz, *J*=7.6 Hz, 1H; C*H*OH), 4.91 (ddt, *J*=8.6 Hz, *J*=4.4 Hz, *J*=3.4 Hz, 1H; C*H*CH_2Cl); ^{13}C NMR (50.3 MHz, $CDCl_3$): δ=32.9, 46.2, 66.9, 76.0, 176.8; IR (KBr): $\tilde{\nu}$= 3408 (m), 2959 (m), 1771 (s), 1186 (s), 1117 (s); MS (EI) m/z (%): 150/152 (1) [M^+], 149/151 (8), 131/133 (10), 101 (27), 73 (100), 57 (82); HRMS (EI): m/z= 150.0060; calc. for $C_5H_7ClO_3$=150.0084.

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31. **161b**+**d** could not be separated and only the NMR-data of **161b** could be reported.
32. Estimated by 1H NMR integration.
33. The fraction, which was used for measuring the rotation of (2*R*,4*S*)-**172b**, had 67 % d.e.
34. The fraction, which was used for measuring the rotation of **172c-d**, had no d.e.

7

Synthetic Studies towards Mureidomycin A and Dihydropyrimidine Nucleosides

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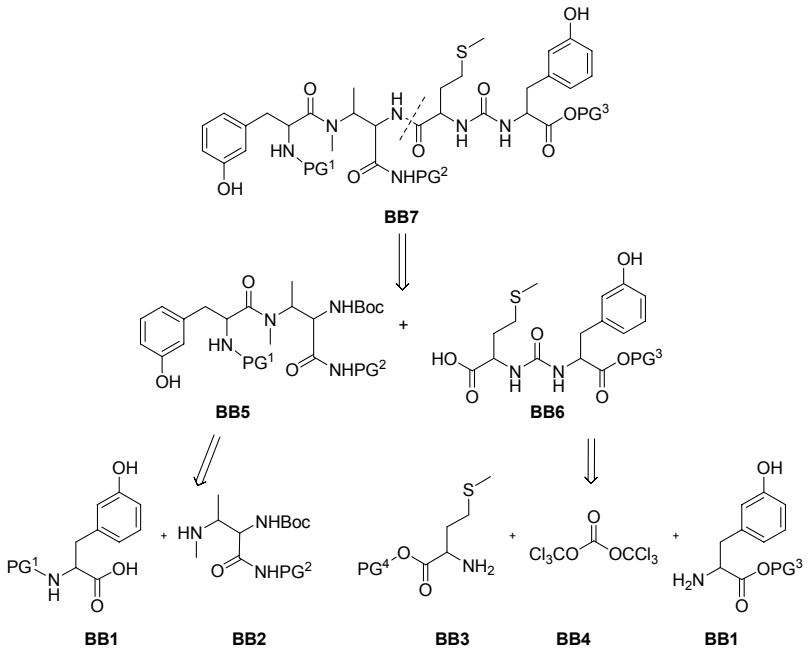
The syntheses of the three building blocks of the peptide backbone of mureidomycin A are described. A model study on the synthesis of dihydropyrimidine nucleosides resulted in the formation of an ortho-ester and a dihydropyrimidine nucleoside, which is connected via *N*3 of the dihydropyrimidine to the ribose moiety.

7.1 Introduction

The synthesis of a small library of dihydropyrimidine (DHPM) nucleosides as potential antibiotic agents was one of the primary goals of this research. In addition, a first study on the total synthesis of mureidomycin A (MRD A) was envisioned. To realise this, the synthesis of the peptide backbone of MRD A is essential. In the first section of this chapter our initial studies to access the required building blocks are described. Further, for the synthesis of the DHPM nucleoside library flexible synthetic methodologies were developed (chapters 3 and 4) for the construction of DHPMs and DHPM analogues. In the second section of this chapter our efforts to prepare nucleosides from riboses and a DHPM-heterocycle as nucleobase are described, which brings the realisation of the DHPM-nucleoside library closer to reality.

7.2 Synthesis of the peptide backbone of mureidomycin A

The synthesis of the peptide backbone of MRD A (BB7) is envisioned as depicted in Scheme 7.1. The synthess of **BB1** (protected *m*-tyrosine), **BB2** (protected 2-amino-3-methylaminobutanoic acid; AMBA) and **BB6** (protected unsymmetrical urea) are described.

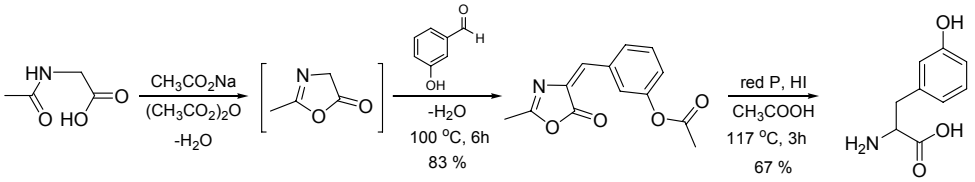


Scheme 7.1 Peptide part of the retrosynthetic analysis of mureidomycin A

7.2.1 Synthesis of unsymmetrical urea (**BB6**)

The unsymmetrical urea moiety in MRD A consists of two different amino acids: *m*-tyrosine and methionine. Although the exact stereochemistry of MRD A is not known, it is assumed that the amino acids have the natural (*S*)-configuration. To confirm this, we wished to develop a general synthetic methodology to access all possible isomers of the unsymmetrical urea.

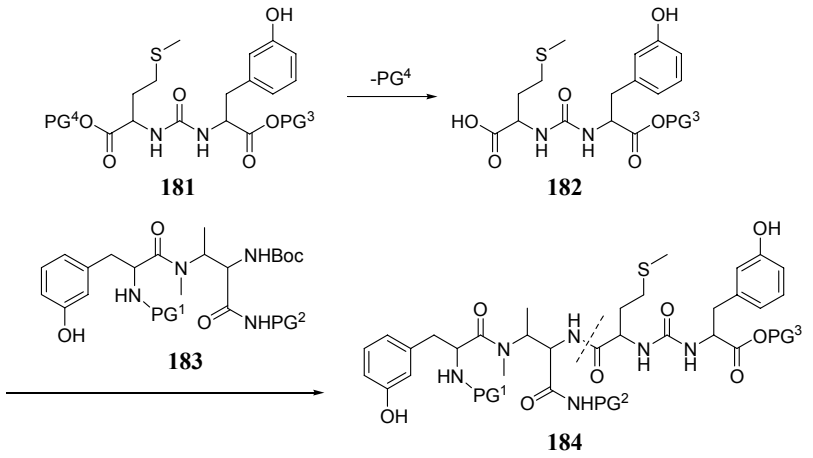
Initially, the unsymmetrical urea from L-methionine and D,L-*m*-tyrosine was prepared. L-methionine is commercially available, while D,L-*m*-tyrosine was easily synthesised via the classical Erlenmeyer condensation reported by Schweet *et al.*[1] Starting with an intramolecular condensation of acetylglycine (**177**) in the presence of acetic anhydride resulted in intermediate **178**, which was subsequently reacted with *m*-hydroxybenzaldehyde to yield azlactone **179** in 83%. This compound can be readily converted to *m*-tyrosine (**180**) by treatment with red phosphorus, hydroiodic acid and acetic acid in 67% yield.[1]



177 **178** **179** **180**

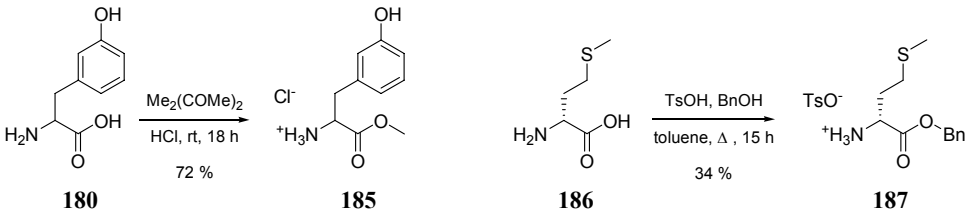
Scheme 7.2 Synthesis of *m*-tyrosine

In general, in the synthesis of unsymmetrical ureas, successful coupling between the amino acids is only efficient when the carboxylic acids are protected as esters. In the literature, different esters are used for this varying from methyl, ethyl to benzyl esters and even the trimethylsilyl protecting group is used.[2] To deduce which protecting group is most suitable for the synthesis of unsymmetrical urea **181**, the final step in the synthesis of the peptide backbone of MRD A has to be considered. During this step, it was planned that, unsymmetrical urea **182** is coupled with the AMBA-derivative **183** (Scheme 7.3). To assure that this reaction will take place at the methionine side, **182** has to be a half acid/half ester urea. Therefore protecting group 4 (PG^4) must be more easily removable than protecting group 3 (PG^3). A procedure that proved successful in the literature is the use of the combination of a benzyl and a methyl-protecting group.[3] The benzyl protecting group can easily be removed by reduction (H_2, Pd/C in methanol), while the methyl ester is unreactive under these conditions. Therefore, it was decided to protect *m*-tyrosine (**180**) as a methyl ester and methionine (**186**) as a benzyl ester.



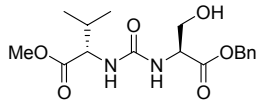
Scheme 7.3 Synthetic plan for peptide backbone of MRD A.

For the protection of *m*-tyrosine as a methyl ester, the procedure of Rachele was followed,[4] using 2,2-dimethoxypropane and HCl resulting in *m*-tyrosine methyl ester hydrochloride salt **185** in 72% yield (Scheme 7.4). L-Methionine is protected as its benzyl ester via a procedure reported by Okada *et al*.[5] Refluxing a mixture of L-methionine (**186**), *p*-toluenesulfonic acid monohydrate, benzyl alcohol in toluene under Dean-Stark conditions resulted in L-methionine benzyl ester *p*-toluenesulfonic acid **187** in 34% yield (Scheme 7.4).



Scheme 7.4 Esterification of the amino acids *m*-tyrosine and L-methionine

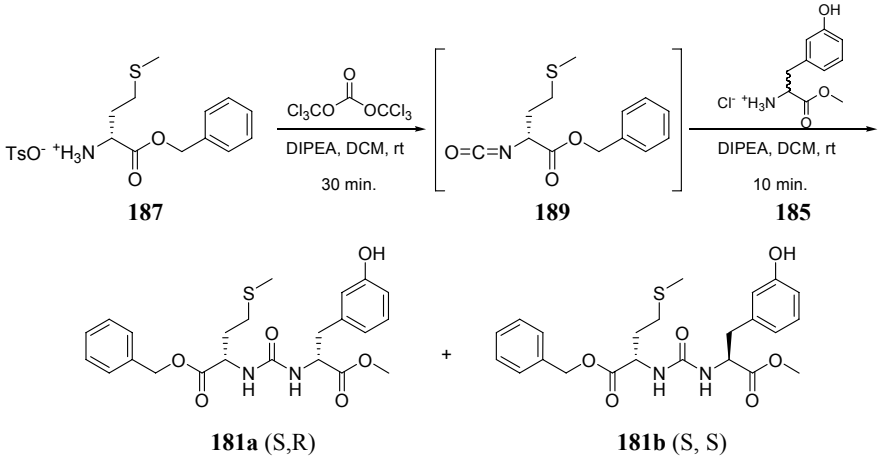
The most direct way to synthesise unsymmetrical ureas starts with phosgene. The use of the very toxic phosgene is highly undesirable, therefore analogues were developed that are less toxic.[6,7] Triphosgene has been used for the synthesis of other unsymmetrical ureas bearing amino acid derivatives.[7] Under mild conditions after a short reaction time, in a facile 'one-pot' fashion unsymmetrical ureas can be synthesised. For example, Randad *et al*. reported the synthesis of **188** with triphosgene as coupling reagent using benzyl- and methyl ester protected amino acids.[7] Therefore, we decided to use this reagent for the synthesis of unsymmetrical urea **181**.



188

Figure 7.1 Unsymmetrical urea synthesised by using triphosgene

L-Methionine benzyl ester *p*-toluenesulfonium salt **187** is reacted with triphosgene under basic conditions resulting in intermediate **189**. Subsequent reaction with the second amino acid *m*-tyrosine methyl ester hydrochloride salt **185** yields unsymmetrical urea **181a-b** in a 1:1 mixture of diastereomers in 70%. These diastereomers can be partially separated by column chromatography. The precise mechanism of the formation of unsymmetrical ureas with triphosgene is not reported in literature. However, it is known that triphosgene converts into phosgene in the presence of a nucleophile.[8] In addition, phosgene is a known reagent for the formation of isocyanates.[9] A possible mechanism is that the methionine benzyl ester *p*-toluenesulfonium salt (**187**) initiates the formation of phosgene. The anionic *p*-toluenesulfonium ion can attack the carbonyl carbon of triphosgene giving tosyl 1,1,1-trichloromethyl carbonate and phosgene. This reaction yields the more nucleophilic chloride ions which can react further with triphosgene to yield more phosgene. The chloride ion is only needed in a catalytic amount, since it is not consumed during the reaction. Subsequently, phosgene can react with methionine benzyl ester, to form the reactive isocyanate **189**, which can react with *m*-tyrosine methyl ester (**185**) yielding the unsymmetrical urea's **181a** and **b**. Thus, with *m*-tyrosine and L-methionine the formation of unsymmetrical urea proved successful. To arrive at the optically pure analogues of **181**, the synthesis of the unsymmetrical urea must be repeated with enantiopure *m*-tyrosine. The synthesis of both enantiomers is currently under investigation.



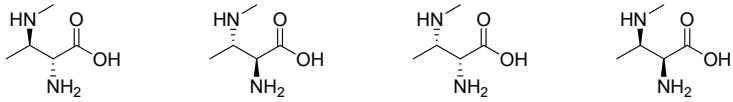
187 **189** **185**

181a (S,R) **181b** (S, S)

Scheme 7.5 Synthesis of unsymmetrical urea **181**

7.2.2 Synthesis of 2-Amino-3-methylaminobutanoic acid (AMBA)

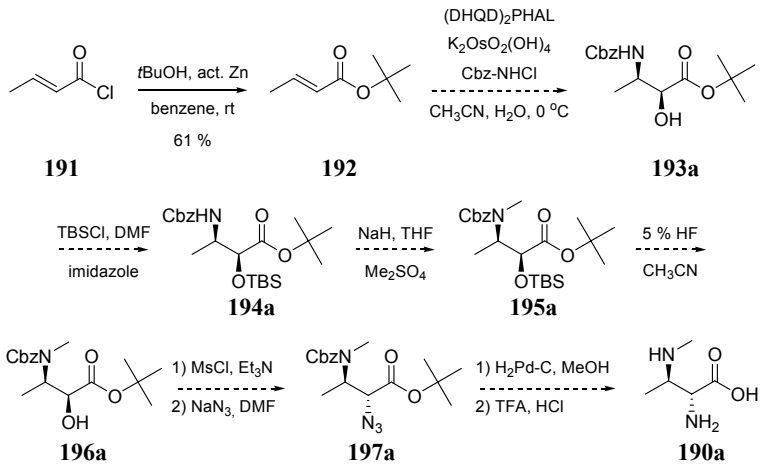
2-Amino-3-methylaminobutanoic acid (AMBA, **190**; Scheme 7.1) is a peptidyl building block that is not only important for the synthesis of MRD A. AMBA is also part of other uridyl peptide antibiotics, like pacidamycins[10] and napsamycins.[11] AMBA is an aza analogue of isoleucine and has been used as an amino acid antagonist (Figure 7.2).[12]



190a (2*R*,3*R*) **190b** (2*S*, 3*S*) **190c** (2*R*, 3*S*) **190d** (2*S*, 3*R*)

Figure 7.2 Possible isomers of AMBA

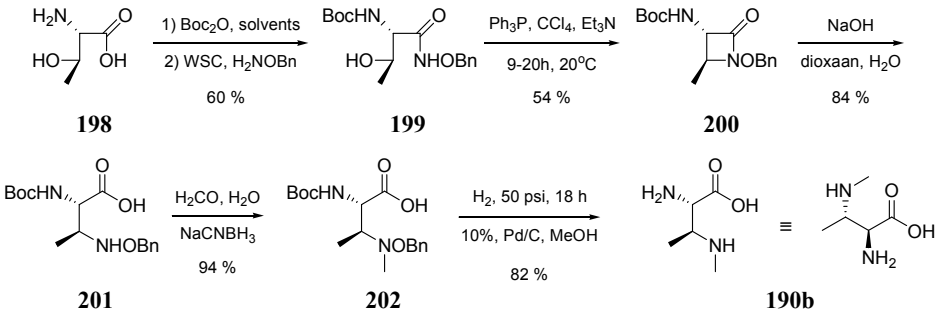
In literature there are two routes reported for the synthesis of AMBA.[3,13] The first reported synthesis employed the method of Janda *et al.*[14] and completed the synthesis starting from the achiral starting material, trans-crotonyl chloride (**191**; Scheme 7.6). This approach is considered very flexible and in principle all four stereoisomers of AMBA (**190**) are accessible (Figure 7.2 and Scheme 7.6).[14,15] In our hands this route was not successful.



Scheme 7.6 Synthesis of AMBA via Janda's route

The first step, synthesis of t-butyl trans crotonyl ester (**192**) from trans crotonyl chloride (**191**) worked in 61% yield, however the purification was troublesome. The large amount of benzene that is necessary for the reaction could not be removed quantitatively by evaporation/distillation without loss of a large amount of product. Several test reactions were carried out with different quantities of benzene, cyclohexene or diethyl ether to

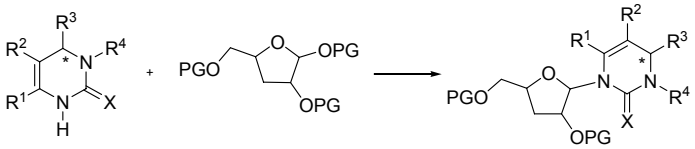
improve the yield, but to no avail. Thus, using cyclohexene as medium, similar purification problems arose as compared to the reaction in benzene. Using only minimal amounts of benzene or diethyl ether, the reaction proved unselective and not quantitative, with yields of only 9% of pure product. Alternative syntheses, like the classical esterification using sulphuric acid[16] or DCC and DMAP[17] did not result in good yields of **192**. The use of a differently substituted crotonyl ester could be envisioned. A benzyl protection group would make synthesis via the same procedure possible, however the synthesis of benzyl trans crotonyl ester was also not quantitative and removal of residual benzyl alcohol led to decomposition of the product. Therefore we turned our attention to the route of Boojamra.[3] He started the synthesis of 2*S*,3*S*-AMBA (**190b**) using the amino acid L-threonine (**198**).[3] This route worked very well and **190b** could be prepared in 20% overall yield in 6 steps. First L-threonine is protected with Boc_2O and converted into the *O*-benzylhydroxamate **199** in 60% yield with (*O*-benzylhydroxyl)-amine and 1-ethyl-3-(3-(dimethyl-amino)propyl)-carbodiimide (WSC). Treatment of **199** with Ph_3P, CCl_4 and Et_3N leads to the hydroxamate nitrogen anion that cyclises, forming **200** in 54% yield.[18] The azetidinone ring in **200** is then hydrolysed using NaOH, providing compound **201** in 84% yield.[3] Then, the *N*-methyl group was introduced by reductive amination with formaldehyde and $NaCNBH_3$ resulting in compound **202** in 94% yield. The last step in this procedure is deprotection of the *N*-methylated amino group. This was done by hydrogenation with H_2, Pd/C to provide the required product **190b** in 82% yield (Scheme 7.7). In the same manner Boojamra *et al*. proved that **190a** can be synthesised from D-threonine.[3] The synthesis of the *syn*-AMBA isomers (**190c-d**) is envisioned starting from the more expensive allo-threonine amino acids.[19]



Scheme 7.7 Synthesis of AMBA according to Boojamra.[3]

7.3 Model study on synthesis of dihydropyrimidine nucleosides

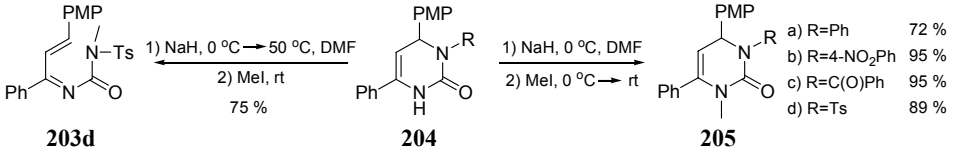
The synthesis of dihydropyrimidine 3'-deoxynucleosides was one of the primary goals of this research (Scheme 7.8). In order to get general insight on the coupling of DHPMs to riboses, we start to investigate whether the free nitrogen (N1) of DHPMs is reactive at all. From bottom-up, we first explored whether DHPMs can be methylated at N1. In order not to use the valuable 3'-deoxy ribose precursor (**176**) we started to examine the coupling of DHPMs with natural riboses.



Scheme 7.8 Coupling of DHPMs to 3'-deoxyriboses

7.3.1 Alkylation of DHPMs

The alkylation of four DHPMs (**204a-d**) was investigated. Via deprotonation of the DHPM with NaH at 0°C and subsequent reaction with methyl iodide, N1-methylated DHPMs (**205a-d**) could be synthesised in good yields (72–95%). In addition, when deprotonation was performed at 50°C with DHPM **204d**, not the N1-methylated DHPM **205d** was formed, but ring opened N3-methylated imine **203d** in 75% isolated yield.



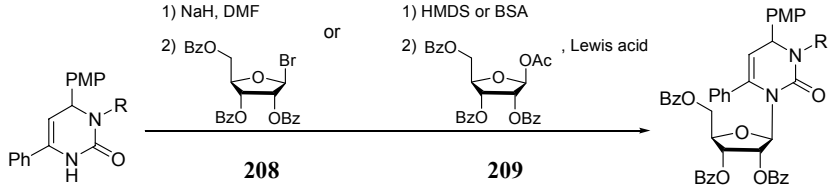
203d **204** **205**

Scheme 7.9 Methylation of DHPMs **204a-d**

Thus, a higher reaction temperature during deprotonation does not lead to a faster reaction, but to ring opening resulting in a more stable anion. Therefore, during the deprotonation the temperature may not exceed room temperature. The high yields of the N1-methylated DHPMs **205a-d** indicate that deprotonation with sodium hydride and subsequent reaction with methyl iodide works very well. It seemed therefore appropriate to react 1'-halogenated riboses deprotonated DHPMs in order to synthesise DHPM the analogous nucleosides.

7.3.2 Coupling of DHPMs to ribose

The synthesis of DHPM nucleosides is envisioned via deprotonation of the base and subsequent reaction with a halogenated ribose or using the Vorbrüggen condensation (Scheme 7.10).[20] Like in the synthesis of N1-methylated DHPMs, the DHPM is first deprotonated with sodium hydride. Subsequent reaction with a brominated ribose should then lead to DHPM-nucleosides. In the Vorbrüggen condensation the DHPM is first silylated, for example with hexamethyldisilazane (HMDS) or bis(trimethylsilyl)acetamide (BSA), which is then followed by Lewis acid catalysed condensation with a protected ribose (Scheme 7.10).



Scheme 7.10 Proposed synthesis of DHPM nucleosides

Under these conditions several attempts to couple DHPM **204b-c** and **206** to riboses **208** and **209** did not result in clear product formation. Table 7.1 summarises the applied conditions and outcome. With LC-MS the correct mass is sometimes observed, however in such a small amount that isolation was not attempted.

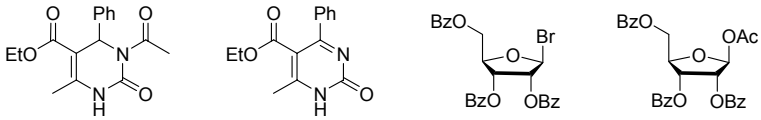
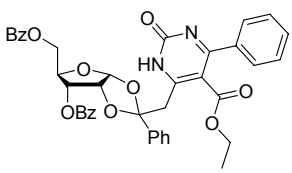
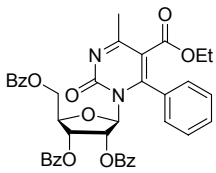


Figure 7.3 Reactants for the coupling reactions

Therefore we decided to use **207** as the heterocyclic base, which is aromatic and in this way resembles more natural nucleobases. First, **207** and **209** are dissolved in dry acetonitrile. BSA is added and the reaction mixture is refluxed for three hours resulting in silylation of **207**. Subsequently, TMSOTf or $SnCl_4$ is added and the reaction mixture is stirred overnight. In the reaction of **207** with $SnCl_4$ all starting ribose **209** was consumed (entry 10; Table 7.1), while with TMSOTf only marginal consumption of **209** was observed (entry 11; Table 7.1). Therefore the reaction of **207** with TMSOTf was heated for an additional 3 hours at 60°C (entry 12; Table 7.1) which resulted in total consumption of **209**. In all reactions of **207** with ribose **209** (entries 10-12; Table 7.1) formation of ortho-ester **210** is observed (Figure 7.4).



210



211

Figure 7.4 Nucleosides **210** and **211**

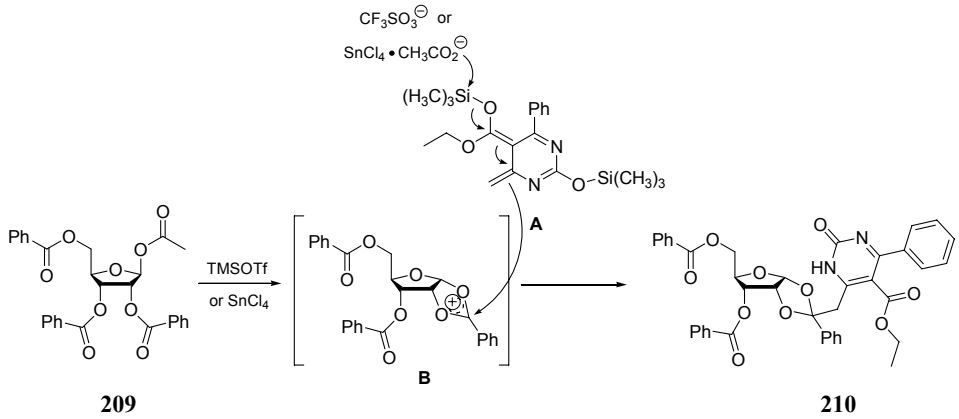
In the $SnCl_4$ mediated reaction **210** was the only product that could be isolated. Although no other product could be identified, only a moderate yield of 36% was achieved. The two TMSOTf mediated reactions also yielded ortho-ester **210**. Depending on the reaction temperature either a very small amount (2%, entry 11; Table 7.1) or a moderate amount (21%, entry 12; Table 7.1) of ortho-ester **210** was formed.

Table 7.1 Attempts to couple DHPMs to riboses

Entry	DHPM	Ribose	Conditions	Results
1	**204b**	**208**	NaH, rt, DMF or CH_3CN	-
2	**204b**	**209**	rt, DCM	-
3	**204b**	**209**	HMDS, $(NH_4)_2SO_4$, DCM, TMSOTf	-[a]
4	**204b**	**209**	NaH, $(NH_4)_2SO_4$, DCM, TMSOTf	-[a]
5	**206**	**209**	HMDS, $(NH_4)_2SO_4$, DCM, TMSOTf	-[b]
6	**206**	**209**	NaH, $(NH_4)_2SO_4$, DCM, TMSOTf	-[b]
7	**204c**	**209**	BSA, CH_3CN, TMSOTf	-[b]
8	**204c**	**209**	NaH, CH_3CN, TMSCl, TMSOTf	-[b]
9	**204c**	**209**	$SnCl_4$	-
10	**207**	**209**	BSA, CH_3CN, $SnCl_4$ (rt)	36% **210**
11	**207**	**209**	BSA, CH_3CN, TMSOTf (rt)	7% **211** + 2% **210**[b]
12	**207**	**209**	BSA, CH_3CN, TMSOTf (60 °C)	21% **210**[c]

a) With LC-MS the correct mass of the nucleoside is observed; b) most of the starting material is recovered; c) 12% of an unknown coupling product was also formed

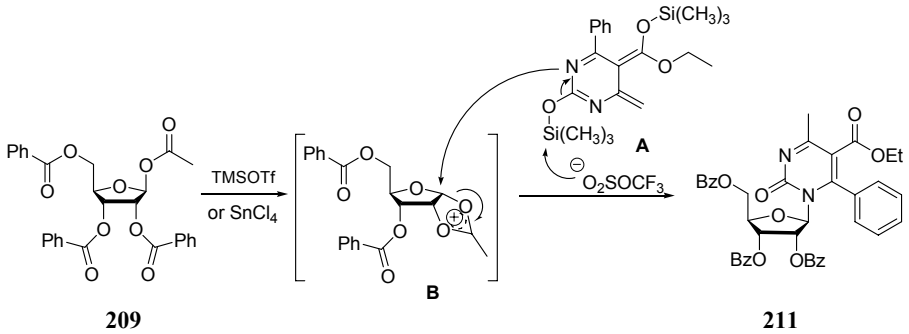
The reaction mediated by TMSOTf at room temperature resulted next to **210** in another coupling product: the nucleoside **211**. Although, a nucleoside analogue, this is not the desired product, since the heterocyclic base is attached via N3 to the ribose. Nevertheless, coupling between a ribose and **207** is achieved via the Vorbrüggen condensation. Besides formation of **210** and **211** predominantly starting material was recovered. Longer reaction times or heating of the reaction mixture could lead to higher yields. Therefore, the coupling reaction using TMSOTf was heated for 3 hours at 60 °C (entry 12; Table 7.1), which resulted in a higher yield of ortho-ester **210**. However, formation of nucleoside **211** was no longer observed. In addition the formation of an unknown coupling product was observed. So far, we were not able to determine the structure of this product, which according to NMR data consists of the nucleobase, the ribose and two TMS-groups. The formation of ortho-ester **210** and nucleoside **211** can be rationalised by the mechanism of the Vorbrüggen condensation (Scheme 7.11 and Scheme 7.12).



Scheme 7.11 Mechanism of formation of ortho-ester **210**

Silylation of **207** with BSA results in intermediate **A**, while reaction of ribose **209** with a Lewis acid ($SnCl_4$ or TMSOTf) results in intermediate **B**. Two different reaction pathways are thus responsible for the formation of ortho-ester **210** and nucleoside **211**. Formation of **210** starts with the attack of the anionic residue of the reaction between the Lewis acid and ribose **209** to the silyl group of intermediate **A**. This results, via isomerisation, in attack of the exocyclic double bond to the ortho-position of intermediate **B**, giving ortho-ester **210** after basic work-up. (Scheme 7.11). The formation of an ortho-ester is a common side reaction of the Vorbrüggen condensation and the formation of an analogous ortho-ester was reported recently.[21] In Scheme 7.12 the mechanism of the formation of nucleoside **211** is

depicted. Attack of the triflate anion on the silyl group and subsequent nucleophilic attack of N3 on the ribose results via basic work-up in nucleoside **211** (Scheme 7.12).



Scheme 7.12 Mechanism of formation of nucleoside **211**

7.4 Conclusion

The synthesis of the protected building blocks of the peptide-backbone of MRD A, *m*-tyrosine **BB1**), AMBA (**BB2**) and unsymmetrical urea (**BB6**) has been accomplished. Protected *m*-tyrosine can be synthesised via the classical Erlenmeyer procedure, protected AMBA via a procedure reported by Boojamra *et al.*[3] in 20% overall yield starting from L-threonine and the unsymmetrical urea (**181**) can be synthesised in a 1:1 diastereomeric mixture in 70% yield. The model study on the synthesis of DHPM nucleosides resulted in the formation of a nucleoside, which is connected via N3 of the pyrimidine to the ribose moiety. Depending on the Lewis acid different side products are formed. Further studies are currently performed to direct this reaction towards the correctly N1-connected nucleoside derivatives.

7.5 Acknowledgements

We thank Dr. Marek Smoluch for measuring the HRMS samples.

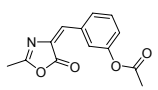
7.6 Experimental section and physical data

7.6.1 General part

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC 200 (200.13 and 50.32 MHz, respectively), a Bruker Avance 250 (250.13 and 62.90, respectively) or a Bruker MSL 400 (400.13 and 100.61 MHz respectively) spectrometer; chemical shifts (δ) are given in ppm, internally referenced to residual solvent resonances (^{1}H: δ 7.29 ppm, ^{13}C: δ 77.0 ppm). Column chromatography was performed with Baker 7024-02 silica gel (40 μ, 60 Å) with cyclohexane (c-

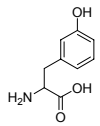
hexane), ethyl acetate (EA), chloroform and methanol as eluents. Thin-layer chromatography (TLC) was performed using silica plates from Merck (Kieselgel 60 F_{254} on aluminium with fluorescence indicator). Compounds on TLC were visualised by UV-detection and/or coloured with anisaldehyde solution followed by heating to 150˚C. High-resolution mass spectra (HRMS, EI) were recorded on a Finnigan Mat 900 spectrometer at 70 eV. IR spectra are recorded on a Mattson-6030 Galaxy spectrophotometer and are reported in cm^{-1}. Melting points were measured on a Stuart Scientific SMP3 melting point apparatus and are uncorrected. Tetrahydrofuran (THF), toluene, acetonitrile and dichloromethane were dried and distilled before use. Sodium hydride was prior to use, washed with dry hexane (2 times 5mM). Commercial chemicals were used as such. Reactions were performed under nitrogen or argon when necessary. AMBA (**190b**),[3] DHPMs **206-207**[22] were synthesised according to literature procedure and NMR data were in accordance with those reported.

7.6.2 Synthetic procedures and Physical Data



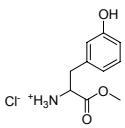
2-Methyl-4-(3'-actetoxybenzal)-5-oxazolone (179)[1]

m-Hydroxybenzaldehyde (8.78 g, 72.0 mmol), acetylglycine (8.42 g, 72.0 mmol), anhydrous sodium acetate (5.91 g, 72.0 mmol) and acetic anhydride (20.4 mL, 216.0 mmol) where thoroughly mixed and heated at 100 °C for 6 h. Hereafter the reaction mixture was allowed to come to room temperature with the resultant formation of a solid mass, at which point 45 mL of ice-cold water was gradually added to the mixture. After storage in the refrigerator (3 ^{o}C) overnight, the product was separated by filtration and thoroughly washed portion wise (4x) with ice-cold water. The obtained air-dried material was a brown solid, which was pure enough to use in the following reaction. Yield: 13.89 g, 60 mmol, 83%; m.p. 119-120 °C [H_2O]; ^{1}H NMR (250.13 MHz, $CDCl_3$): δ=2.33 (s, 3H; CH_3CO), 2.41 (s, 3H; CH_3CN), 7.10 (s, 1H; C*H*=), 7.17 (ddd, *J* = 8.2 Hz, *J* = 2.4 Hz, *J* = 1.0 Hz, 1H; Ph-*H*), 7.45 (dd, *J* = 8.0 Hz, *J* = 7.9 Hz, 1H; Ph-*H*), 7.85 (dt, *J* = 7.8 Hz, *J* = 1.0 Hz, 1H; Ph-*H*), 7.92–7.94 (m, 1H; Ph-*H*); ^{13}C NMR (62.90 MHz, $CDCl_3$): δ=15.1, 20.1, 124.4, 124.7, 129.7 (2C), 130.0, 133.3, 134.5, 150.9, 166.6, 167.5, 169.2; IR (KBr): $\tilde{\nu}$=3431 (w), 1804 (s), 1763 (s), 1657 (s), 1597 (w), 1213 (s), 1167 (s).



***m*-Tyrosine (180)**[1]

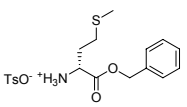
Azlactone (**179**) (10.03 g, 43 mmol) was mixed with glacial acetic acid (54 mL), hydroiodic acid (54 mL) and red phosphorus (4.00 g, 129 mmol) and heated at reflux temperature (117 ^{o}C) for 3 h. The hot mixture was filtered over a 3-cm plug of silica, after which the solvents were evaporated. To remove the remaining hydroiodic acid, three times 4 mL water was added and evaporated. The remaining solid amino acid hydroiodide was dissolved in 25 mL hot water and treated with norit. The mixture was neutralised with concentrated ammonia to a pH of 5.0 to 5.5. Absolute ethanol (20 mL) was added and the mixture was allowed to stand overnight in the refrigerator (3 ^{o}C). The obtained brown solid was filtered and dried. Yield: 5.25 g, 29 mmol, 67%; analytical data were in accordance with literature data.



***m*-Tyrosine methyl ester hydrochloride (185)**[4]

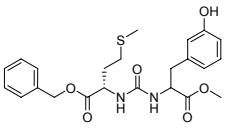
m-Tyrosine (**180**) (0.37 g, 2.0 mmol) was suspended in 2,2-dimethoxypropane (25 mL) and concentrated hydrochloric acid (2 mL) was added. The mixture was kept at room temperature overnight resulting in a dark brown solution. The solvents were evaporated and the residue was dissolved in warm THF. Overnight standing in the refrigerator (3 ^{o}C) resulted in *m*-tyrosine methyl ester hydrochloride as brown crystals. Yield: 0.34 g,

1.5 mmol, 73%; ^{1}H NMR (250.13 MHz, D_2O): δ=3.23 (dd, *J* = 14.5 Hz, *J* = 7.5 Hz, 1H; CH_2), 3.29 (dd, *J* = 14.5 Hz, *J* = 6.0 Hz, 1H; CH_2), 3.88 (s, 3H; CH_3), 4.46 (dd, *J* = 7.2 Hz, *J* = 6.0 Hz, 1H; C*H*N), 6.81 (s, 1H; Ph-*H*), 6.85–6.91 (m, 2H; Ph-*H*), 7.31 (dd, *J* = 8.0 Hz, *J* = 7.8 Hz, 1H; Ph-*H*); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ= 35.9, 54.0, 54.4, 115.4, 116.8, 121.8, 131.1, 136.0, 156.5, 170.5.



L-Methionine Benzyl ester p-toluenesulfonate (187)[23]

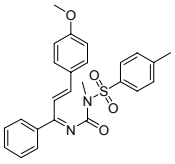
A mixture of L-methionine (7.02 g, 46.9 mmol), *p*-toluenesulfonic acid monohydrate (11.60 g, 61.0 mmol), benzyl alcohol (25 mL, 234.5 mmol) and toluene (50 mL) was refluxed for 5 h under Dean-Stark conditions to remove all water that is formed during the reaction. Then, the solution was concentrated and the organic layer was extracted three times with water (50 mL). The combined water layer was washed three times with 90 mL diethyl ether after which the pH was adjusted to 10 with 20% Na_2CO_3. The resulting precipitate was extracted with ethyl acetate (3×90 mL) and the ethyl acetate layer was dried with Na_2SO_4. The solvent was evaporated and the resulting syrupy residue was dissolved in diethyl ether. A saturated ethereal solution of *p*-toluenesulfonic acid was added and the resulting precipitate was collected by filtration washed with diethyl ether and dried. More product precipitated by allowing the first organic layer to stand, resulting in L-methionine benzyl ester p-toluenesulfonate as a white solid. Yield: 6.61 g, 16 mmol, 34%; $[\alpha]^{34}_D$ = + 1.8 (c = 1.1 in MeOH); m.p. 127-129 °C; ^{1}H NMR (250 MHz, $CDCl_3$): δ=1.85 (s, 3H; Ph-CH_3), 2.10–2.14 (m, 2H; CH_2CH_2S), 2.31 (s, 3H; SCH_3), 2.42–2.47 (m, 2H; CH_2S), 4.14 (t, *J* = 6.0 Hz, 1H; C*H*N), 5.02 (d, *J* = 12.3 Hz, 1H; CH_2O), 5.14 (d, *J* = 12.3 Hz, 1H; CH_2O), 7.09 (d, *J* = 7.8 Hz, 2H; *m*-C*H*(Ts)), 7.25–7.29 (m, 5H; Ph-*H*)), 7.74 (d, *J* = 7.8 Hz, 2H; *o*-C*H*(Ts)), 8.35 (br s, 3H; NH_3); ^{13}C NMR (62.9 MHz, $CDCl_3$): δ=14.8, 21.3, 29.0, 29.5, 52.2, 67.9, 126.1 (2C), 128.5 (5C), 128.9 (2C), 134.7, 140.4, 141.3, 169.0; IR (KBr): $\tilde{\nu}$= 3158 (w), 3034 (w), 2918 (w), 1746 (s), 1524 (s), 1213 (s), 1125 (s), 1036 (s).



Unsymmetrical urea 181

Triphosgene (0.11 g, 0.37 mmol) was dissolved in dry CH_2Cl_2 (2 mL) under a dry argon atmosphere. A mixture of L-methionine benzyl ester p-toluenesulfonate salt (**187**) (0.17 g, 1 mmol) and diisopropyl-ethylamine (DIPEA, 0.38 mL, 2.2 mmol) in CH_2Cl_2 (3.5 mL) was slowly added to the stirred solution of triphosgene over a period of 30 min at room temperature using a syringe pump. After a further 5 min stirring, a solution of (D,L)-m-tyrosine methyl ester hydrochloride (**185**) and DIPEA (0.38 mL, 2.2 mmol) in CH_2Cl_2 (2 mL) was added in one portion. The reaction mixture was stirred for an additional 10 min at room temperature, evaporated to dryness, solved in ethyl acetate, washed with 10% aqueous $KHSO_4$ (5 mL), 5% aqueous $NaHCO_3$ (5 mL), and brine (5 mL), dried over $MgSO_4$, and evaporated to give the crude product. In the first experiment purification by CC (methanol/$CHCl_3$ 1:100) yielded a 1:1 mixture of diastereomers in 70% yield. Data of 1:1 mixture of diastereomers: ^{1}H NMR (400.13 MHz, $CDCl_3$): δ=1.71–2.12 (m, 4H; CH_2CH_2S), 1.96 (s, 3H; SCH_3), 2.00 (s, 3H; SCH_3), 2.38–2.47 (m, 4H; CH_2S), 2.90–3.19 (m, 4H; CH_2CHCO$_2$Me), 3.65 (s, 3H; CO$_2$CH_3), 3.70 (s, 3H; CO$_2$CH_3), 4.52–5.21 (m, 8H; C*H*(CH$_2$)$_2$S, C*H*CO$_2$Me and CH_2Ph), 5.50–5.78 (m, 4H; N*H*); 6.46–6.73 (m, 6H; Ph-*H*), 7.02–7.12 (m, 2H; Ph-*H*), 7.31–7.51 (m, 10H; CH$_2$Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=15.4 (2C), 29.9, 30.0, 31.9, 32.1, 37.9, 38.7, 52.2 (2C), 52.4 (2C), 53.5, 54.0, 67.5, 68.1, 114.4, 114.7, 116.3, 116.5, 120.4, 121.2, 128.3 (2C), 128.5 (4C), 128.6 (2C), 128.7 (2C), 129.8 (2C), 134.8, 135.1, 136.9, 137.7, 156.3, 156.9 (2C), 157.7, 172.4 (2C), 173.4 (2C).
In a second experiment purification by CC (methanol/$CHCl_3$ 1:100) yielded one of the two isomers in 30% yield. Data of one isomer; ^{1}H NMR (200.13 MHz, $CDCl_3$): δ=1.83–2.03 (m, 2H; CH_2CH_2S),

2.00 (s, 3H; SCH_3), 2.40–2.50 (m, 2H; CH_2S), 3.02–3.08 (m, 2H; CH_2CHCO$_2$Me), 3.66 (s, 3H; CO$_2$CH_3), 4.57 (dt, *J*=7.6 Hz, *J*=5.1 Hz, 1H; C*H*(CH$_2$)$_2$S), 4.72 (dt, *J*=7.7 Hz, *J*= 6.2 Hz, 1H; C*H*CO$_2$Me), 5.07 (d, *J*=12.2 Hz, 1H; CH_2Ph), 5.15 (d, *J*=12.2 Hz, 1H; CH_2Ph), 5.26 (d, *J*=7.9 Hz, 1H; N*H*), 5.54 (d, *J*=7.7 Hz, 1H; N*H*), 6.60–6.62 (m, 2H; Ph-*H*), 6.68-6.70 (m, 1H; Ph-*H*), 7.10 (dd, *J*=7.6 Hz, *J*=7.8 Hz, 1H; Ph-*H*), 7.30–7.45 (m, 5H; CH$_2$Ph-*H*); ^{13}C NMR (62.90 MHz, CDCl$_3$): δ=15.3, 29.8, 32.2, 38.1, 52.3, 52.5, 54.3, 67.4, 114.3, 116.4, 121.0, 128.4 (2C), 128.5, 128.6 (2C), 129.6, 135.2, 137.5, 156.5, 157.0, 173.1, 173.3.

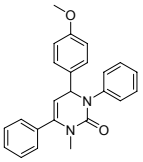


1-(3-(4-methoxyphenyl)-1-phenylallylidene)-3-methyl-3-tosylurea (203)

DHPM **204d** (0.152 g, 0.35 mmol) is added to a suspension of sodium hydride (0.013 g, 0.53 mmol) in DMF (2 mL) at 0°C. The suspension is stirred for 30 min at 0°C, 2 h at room temperature and 1 h at 50°C. After cooling down to room temperature, methyl iodide (0.043 mL, 0.69 mmol) is added and the reaction mixture is stirred overnight at room temperature. The crude product is concentrated under reduced pressure and purified by CC (c-hexane/EA 4:1), which resulted in a light yellow oil of **203**. Yield: 75%; ^{1}H NMR: (250.13 MHz, CDCl$_3$): δ=3.46 (s, 3H, NCH_3), 3.87 (s, 3H; CH_3O), 6.83–7.80 (m, 15H; Ph-*H* and C*H*=C*H*); ^{13}C NMR (100.61 MHz, CDCl$_3$): δ=21.6, 33.3, 55.4, 114.3 (2C), 127.5, 128.1 (2C), 128.3 (2C), 129.0 (2C), 121.3, 129.3 (2C), 129.9 (2C), 130.7, 136.4, 136.5, 144.3, 145.3, 160.5, 161.5.

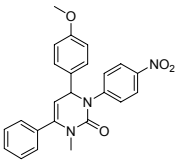
General procedure for the synthesis of N1-methylated DHPMs 205a-d

To a suspension of sodium hydride (1.0 equiv) in DMF (0.08 M) at 0°C DHPM **204** (1 equiv) is added. The suspension is stirred for 75 min at 0°C. Then methyl iodide (1 equiv) is added and the solution is allowed to warm-up overnight. The crude product is concentrated under reduced pressure and purified by CC (c-hexane/EA 4:1).



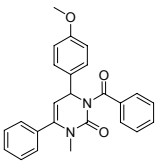
4-(4-methoxyphenyl)-1-methyl-3,6-diphenyl-3,4-dihydropyrimidin-2(1H)-one (205a)

Yield: 72%; ^{1}H NMR: (250.13 MHz, CDCl$_3$): δ=3.06 (s, 3H; CH_3N), 3.83 (s, 3H; CH_3O), 5.20–5.27 (m, 2H; C*H*), 6.88 (d, *J*=8.6 Hz, 2H; Ph-*H*), 7.14–7.40 (m, 12H; Ph-*H*); ^{13}C NMR (100.61 MHz, CDCl$_3$): δ=33.3, 55.2, 62.3, 105.0, 114.1 (2C), 126.2, 127.0 (2C), 127.8 (2C), 128.3 (2C), 128.4 (2C), 128.6, 128.7 (2C), 134.3, 135.4, 140.4, 142.4, 154.6, 159.2; HRMS: *m/z*=370.1691 [M]$^+$, calc. for $C_{24}H_{22}N_2O_2$=370.1681.



4-(4-methoxyphenyl)-1-methyl-3-(4-nitrophenyl)-6-phenyl-3,4-dihydro-pyrimidin-2(1H)one (205b)

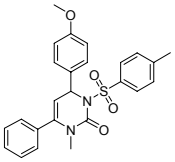
Yield: 95%; ^{1}H NMR: (250.13 MHz, CDCl$_3$): δ=3.06 (s, 3H; CH_3N), 3.82 (s, 3H; CH_3O), 5.34–5.40 (m, 2H; C*H*), 6.92 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.19–7.44 (m, 9H; Ph-*H*), 8.16 (d, *J*=7.2 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, CDCl$_3$): δ=33.5, 55.3, 60.7, 105.6, 114.6 (2C), 124.2 (2C), 125.1 (2C), 126.9 (2C), 128.1 (2C), 128.5 (2C), 128.9 (2C), 133.0, 134.5, 140.3, 144.4, 148.4, 154.3, 159.4; HRMS: *m/z*=415.1531 [M]$^+$, calc. for $C_{24}H_{21}N_3O_4$=415.1532.



3-benzoyl-4-(4-methoxyphenyl)-1-methyl-6-phenyl-3,4-dihydropyrimidin-2(1H)-one (205c)

Yield: 95%; ^{1}H NMR: (250.13 MHz, CDCl$_3$): δ=3.00 (s, 3H; CH_3N), 3.84 (s, 3H; CH_3O), 5.79 (d, *J*=7.0 Hz, 1H; C*H*C), 6.02 (d, *J*=7.0 Hz, 1H; C*H*N), 6.95 (d, *J*=6.8

Hz, 1H; Ph-*H*), 7.42–7.66 (m, 12H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=33.6, 53.2, 55.2, 109.0, 114.2 (2C), 127.6 (2C), 127.9 (2C), 128.1 (2C), 128.2 (2C), 128.7 (2C), 129.2, 131.1 (2C), 134.2, 136.3, 142.0, 153.6, 159.4, 171.5; HRMS: *m/z*=398.1631 $[M]^+$, calc. for $C_{25}H_{22}N_2O_3$=398.1630.

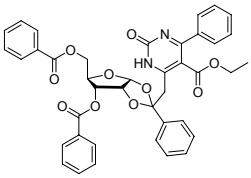


3,4-dihydro-4-(4-methoxyphenyl)-1-methyl-6-phenyl-3-tosylpyrimidin-2(1H)-one (205d)

Yield: 85%; ^{1}H NMR: (250.13 MHz, $CDCl_3$): δ=2.94 (s, 3H; C*H*$_3$N), 3.87 (s, 3H; C*H*$_3$O), 5.42 (d, *J*=6.9 Hz, 1H; C*H*C), 6.07 (d, *J*=6.9 Hz, 1H; C*H*N), 6.89 (d, *J*=8.7 Hz, 2H; Ph-*H*), 7.14 (d, *J*=8.2 Hz, 2H; Ph-*H*), 7.26–7.53 (m, 9H; Ph-*H*); ^{13}C NMR (62.90 MHz, $CDCl_3$): δ=21.6, 33.2, 55.4, 57.1, 107.2, 114.4 (2C), 128.2 (2C), 128.5 (2C), 128.6 (2C), 128.8 (2C), 128.9 (2C), 129.1, 132.4, 134.2, 139.9, 144.0, 151.3, 159.8.

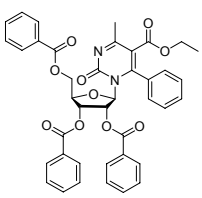
Procedure for pyrimidine nucleoside synthesis

Ribose **209** (0.13 g, 0.26 mmol) and pyrimidine **207** (0.20 g, 0.78 mmol) are co-evaporated three times with anhydrous CH_3CN (3 mL) under argon. Dry CH_3CN (3 mL) and BSA (0.19 mL, 0.78 mmol) are added at room temperature. The mixture was stirred at 65°C for three h. After cooling down to room temperature, TMSOTf or $SnCl_4$ (1.5 equiv) is added and the reaction mixture is stirred overnight at room temperature. One of the reactions with TMSOTf (entry 12; Table 7.1) is heated three more hours at 60°C. The other two reactions are worked up immediately via the following procedure: the reaction mixture is diluted with DCM (20 mL) and washed with sat. $NaHCO_3$ sol. (10 mL) and water (2×10 mL). The combined organic layer is dried over Na_2SO_4 and concentrated under reduced pressure. The crude product is purified by CC (c-hexane/EA 2:1 → EA).



Ortho ester 210

^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=0.77 (t, *J*=7.1 Hz, 3H; C*H*$_3$), 3.45–3.49 (m, 1H; *H*-4'), 3.57 (d, *J*= 13.7 Hz, 1H; C*H*$_2$CN), 3.61 (d, *J*= 13.7 Hz, 1H; C*H*$_2$C), 3.80 (dd, *J*=7.1 Hz, *J*=10.8 Hz, 1H; C*H*$_2$CH$_3$), 3.88 (dd, *J*=7.1 Hz, *J*=10.8 Hz, 2H; C*H*$_2$CH$_3$), 4.09 (dd, *J*=4.1 Hz, *J*=12.3 Hz, 1H; *H*-5'), 4.31 (dd, *J*=3.3 Hz, *J*=12.3 Hz, 1H; *H*-5'), 4.85 (dd, *J*=5.5 Hz, *J*=9.3 Hz, 1H; *H*-3'), 5.09 (dd, *J*=4.2 Hz, *J*=5.5 Hz, 1H; *H*-2'), 6.03 (d, *J*=4.1 Hz, 1H; *H*-1'), 7.26–7.59 (m, 16H; Ph-*H*), 7.83 (d, *J*= 7.8 Hz, 2H; Ph-*H*), 7.90 (d, *J*= 7.7 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=13.2, 44.0, 61.5, 62.7, 72.8, 76.5, 79.1, 105.6, 112.8, 113.8, 125.8 (2C), 128.0 (2C), 128.1 (2C), 128.3 (4C), 128.5 (2C), 128.8, 128.9, 129.5, 129.6 (2C), 129.9 (2C), 130.7, 133.1, 133.6, 141.0, 157.1, 165.2, 165.8, 166.1;[24] MS (EI) m/z (%): 322 (12), 218 (36), 105 (100), 77 (50).



Nucleoside 211

^{1}H NMR: (400.13 MHz, $CDCl_3$): δ=0.81 (t, *J*=7.1 Hz, 3H; C*H*$_3$CH$_2$), 2.52 (s, 3H; C*H*$_3$), 3.86 (q, *J*=7.1 Hz, 2H; C*H*$_2$), 4.54–4.60 (m, 1H; *H*-4'), 4.69–4.90 (m, 2H; *H*-5'), 5.44 (d, *J*=2.2 Hz, 1H; *H*-'1), 6.15 (dd, *J*=7.1 Hz, *J*=8.1 Hz, 1H; *H*-'3), 6.23 (dd, *J*=2.2 Hz, *J*=7.1 Hz, 1H; *H*-'2), 7.20–7.52 (m, 14H; Ph-*H*), 7.68 (d, *J*=7.2 Hz, 2H; Ph-*H*), 7.90 (d, *J*=7.6 Hz, 2H; Ph-*H*), 8.06 (d, *J*=7.3 Hz, 2H; Ph-*H*); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ=13.4, 24.7, 61.4, 64.3, 72.4, 74.3, 80.5, 94.0, 113.8, 128.2–133.4 (24C)[25], 153.6, 157.7, 165.0, 165.4, 165.5, 166.3, 174.3; MS (EI) m/z (%): 445 (10), 259 (11), 218 (10), 105 (100).

7.7 References & Notes

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24. Three C_q singnals are not observed due to isomerisation of the nucleobase.
25. In this region are all aromatic C-signals observed.

Summary and Outlook

The naturally occurring mureidomycin A (MRD A) shows potent antibiotic activity and is up to now only isolated from the bacterium *Streptomyces flavidovirens* SANK 60486. It is built-up from two moieties: a 3'-deoxy-nucleoside moiety, which is linked via an unusual enamide bond to a peptide moiety. This peptide consists of non-natural amino acids, like *m*-tyrosine and 2-amino-3-methylaminobutanoic acid (AMBA) and possesses also an unsymmetrical urea function (Figure 8.1). In chapter 1 we summarised structure-activity relationship (SAR) studies and the possible mode of action of mureidomycin A. For more dedicated biological studies we decided to synthesise analogous dihydropyrimidine nucleosides (**A**) that include the unusual enamide bond (Figure 8.1). These nucleoside analogues themselves could be biologically active as well. An overview of a large range of biologically active nucleoside analogues with 5-or 6-membered nucleobases was discussed in chapter 2. Many therapeutic activities are indeed connected to these nucleoside analogues, which stimulated our further research in this area.

The synthesis of an analogue of MRD A, dihydropacidamycin D, showed that this type of uridyl peptide compounds without the enamide bond, can be obtained from chiral pool starting materials.[1] For example, the 3'-deoxyuridine moiety can be prepared via a rather lengthy route starting from uridine. However, we aim at a more versatile approach for the synthesis of dihydropyrimidine nucleosides **A**. Therefore, disconnection between the sugar and the base resulting in 3'-deoxyribose moiety **B** and dihydropyrimidine (DHPM) **C** seemed appropriate (Figure 8.1).

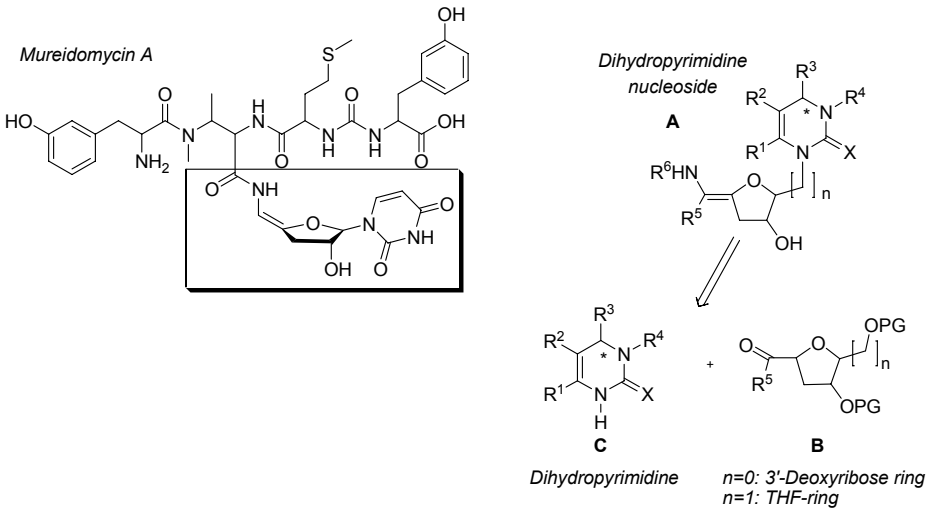
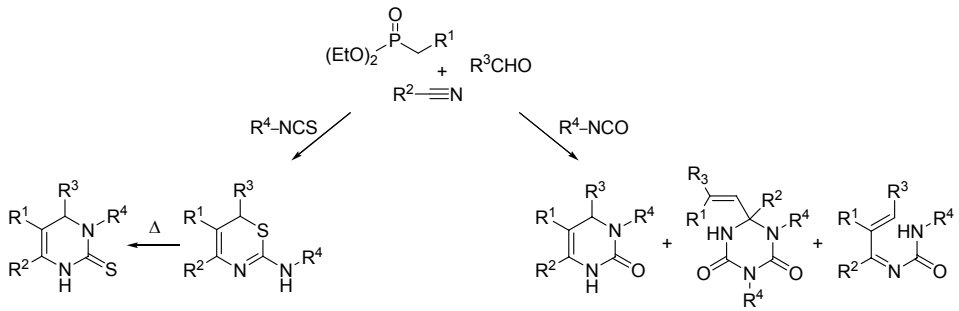


Figure 8.1 Mureidomycin A and dihydropyrimidine nucleosides

In order to prepare these moieties a stereoselective chemo-enzymatic cascade reaction for the synthesis of 3'-deoxyribose moiety **B** and a multi-component approach for the synthesis of dihydropyrimidines **C** were investigated in detail (chapters 3-6).

The synthesis of dihydropyrimidines **C** can be achieved via a four-component reaction (4CR) of phosphonates, nitriles, aldehydes and isocyanates under basic conditions. The scope and limitations of this reaction have been explored. While the nitrile and aldehyde input can be varied extensively, the phosphonate input is rather restricted. When phosphonates other than diethyl methylphosphonate are used, phosphoramidates are observed predominantly. However, differently substituted phosphonates may lead to other C5-functionalised DHPMs, which can be used for further manipulations. Further research in this direction is currently in progress in our group. Variation of the isocyanate input results in the formation of several products: dihydropyrimidines, imines and triazinane diones (Scheme 8.1). The application of a chiral aldehyde led to the diastereoselective formation of a dihydropyrimidine in over 80 % *de*, which is promising for a first small study. Chiral inputs with the stereocenter close to the newly formed stereocenter may be better candidates to induce chirality. Furthermore, the application of chiral catalysis is another possibility of introducing chirality.

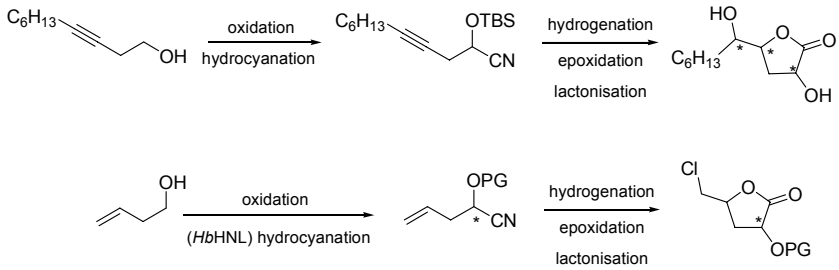
The synthesis of thio-dihydropyrimidines **C** was envisioned via the same 4CR using isothiocyanates as input. However under the conditions of the 4CR, thiazines were formed (Scheme 8.1). In a study thiazines with or without an ester functionality at C5 showed easy isomerisation to thio-dihydropyrimidines under microwave heating. Whereas the thiazines without an ester function already rearranged at 120°C, the thiazines with an ester group did so at a considerably higher temperature of 210°C.



Scheme 8.1 Different outcomes of 4CR

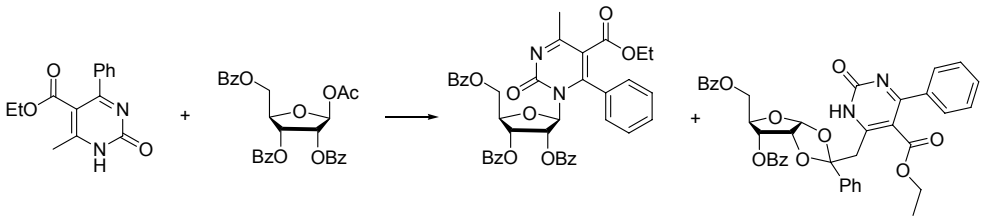
The synthesis of the second part of dihydropyrimidine nucleoside **A**, the 3'-deoxyribose moiety **B**, was planned via a *de novo* chemo-enzymatic cascade starting from simple alcohols. First a model study has been performed resulting in 3'-deoxy-5'-hexylribolactone

in 44 % overall yield starting from 3-decyn-1-ol (Scheme 8.2). Analogous treatment of 3-buten-1-ol resulted in 3'-deoxy-5'-chlororibolactone. The use of HCl in the last step, the hydrolysis induced lactonisation, led to the formation of a 5'-chlorolactone, which makes further synthetic manipulations easier since differentiation between the 2'- and 5'-position is already realised in this way (Scheme 8.2). Finally, the route was adapted to a chemo-enzymatic route, which introduces the stereocenter at C2. A general protocol was developed for the oxidation-hydrocyanation of γ,δ-unsaturated alcohols using (immobilised) TEMPO/PhI(OAc)$_2$ in combination with *Hevea Brasiliensis* hydroxynitrile lyase (*Hb*HNL) and led to high *ee*'s of the cyanohydrin precursors. To finish the synthesis of the 3'-deoxyribose moiety **B** the 3'-deoxy-5'-chlorolactone must be reduced; similar reductions have been reported using DIBAL.[2]



Scheme 8.2 Synthesis of 3'-deoxylactones

To fulfil the primary goal of this research, the synthesis of dihydropyrimidine nucleosides **A**, a model study on the coupling of protected natural riboses to our dihydropyrimidines was performed. Both the Vorbrüggen condensation and a nucleophilic substitution did not give the desired nucleoside yet. The use of a close, but aromatic, pyrimidine analogue as the base (Scheme 8.3) in the Vorbrüggen condensation did result in the formation of a nucleoside, which is connected via N3 to the ribose moiety (Scheme 8.3). In addition, depending on the Lewis acid different side products were formed (Scheme 8.3). The formation of the ortho-ester may be prevented by the use of a differently protected ribose, for example a pivaloyl-protected ribose. Furthermore, the application of different equivalents, solvent, temperature or Lewis acid could promote formation of dihydropyrimidine nucleosides **A**. Currently, further experiments to achieve this are being performed.



Scheme 8.3 Vorbrüggen condensation of pyrimidine with ribose

Finally, studies toward the total synthesis of mureidomycin A were initiated. The synthesis of the building blocks of the peptide backbone has been accomplished. In the near future the building blocks will be linked, which should be straightforward and will be done via standard peptide coupling. Still our original strategy holds to first synthesise the complete peptide moiety and the 3'-deoxuridine and in the final step generate the enamide linkage, since this enamide linkage is the most sensitive part of mureidomycin A.

The synthesis of the enamide linkage is planned via coupling of a vinyl iodide nucleoside with an amide, which is catalysed by Cu(I) in the presence of base, a promoter in a polar aprotic solvent.[3] Alternatively, the method of Bugg *et al*,[4] using an aza-Wittig reaction for the synthesis of the enamide linkage can be considered. However in this method no selective *E*- or *Z*- enamide is formed and the yield is rather low (13 %). Our future attention will be focused on the synthetic methodology to synthesise the crucial enamide function in MRD A and analogues.

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Samenvatting

De eerste antibiotica werden ongeveer vijfenzeventig jaar geleden ontwikkeld. Dit zorgde er voor dat tot dan toe ongeneeslijke ziekten behandeld konden worden. In de loop der jaren echter hebben misbruik en verkeerde doseringen van antibiotica geleid tot resistente bacteriën. Hierdoor is er een vraag ontstaan naar nieuwe antibiotica. Deze kunnen worden ontwikkeld door de modificatie van bestaande middelen, maar een mogelijk betere aanpak is de ontwikkeling van antibiotica met een nieuw werkingsmechanisme. Een voorbeeld van een potentieel antibioticum met een nieuw werkingsmechanisme is Mureidomycine A.

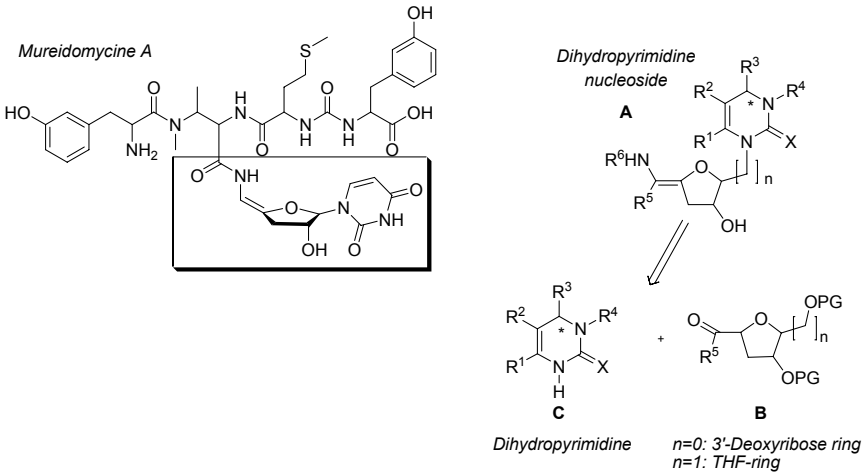
De natuurstof Mureidomycine A (MRD A) bezit een hoge antibiotische activiteit die waarschijnlijk kan worden teruggevoerd op de bijzondere chemische structuur. MRD A bestaat uit twee delen, namelijk een 3'-deoxynucleoside deel dat via een ongebruikelijk enamide binding verbonden is met een peptide deel. Het peptide deel bevat naast een opvallende niet symmetrische ureum functionaliteit o.a. de niet proteogene aminozuurresiduen *m*-tyrosine en 2-amino-3-methylaminobutaan zuur (AMBA). In hoofdstuk 1 worden de verschillende studies naar de structuur-activiteits relatie en het werkingsmechanisme van mureidomycine A samengevat.

Tot nu toe kon MRD A slechts geïsoleerd worden uit de bacterie *Streptomyces flavidovirens* SANK 60486. Om zelf gerichtere biologische studies te kunnen doen, is besloten om analoge dihydropyrimidine nucleosiden (**A**) te synthetiseren op basis van de bijzondere enamide binding (Figuur 8.1). Daarbij is het niet ondenkbaar dat deze nucleoside analoga mogelijk zelf ook een biologische activiteit bezitten. Om dit te onderzoeken wordt in hoofdstuk 2 een literatuurstudie beschreven naar biologisch actieve nucleoside analoga die opgebouwd zijn uit een vijf- of zes-ring nucleobase die via het stikstofatoom aan een ribose suikereenheid zijn verbonden. Het enorme spectrum aan verbindingen en bijbehorende biologische activiteiten die dit opleverde, vormde een stimulans voor de voortzetting van het onderzoek.

De synthese van dihydropacidamycine D, een analoog van MRD A, zonder de karakteristieke enamide binding, laat zien dat dit type verbindingen verkregen kan worden uit de koppeling van de verschillende afzonderlijke chirale bouwstenen. Zo werd bijvoorbeeld de 3'-deoxyuridine functie via een relatief lange route gesynthetiseerd uit het nucleoside uridine.[1]

In dit onderzoek wordt getracht dihydropyrimidine nucleosiden **A** via een veelzijdigere benadering te synthetiseren. Hiervoor wordt het nucleoside **A** opgedeeld in een 3'-deoxyribose fragment **B** en een dihydropyrimidine (DHPM) fragment **C**. Getracht wordt het 3'-deoxyribose fragment **B** via een stereoselective chemo-enzymatische cascade reactie te synthetiseren en voor de synthese van het dihydropyrimidine (DHPM) fragment **C**

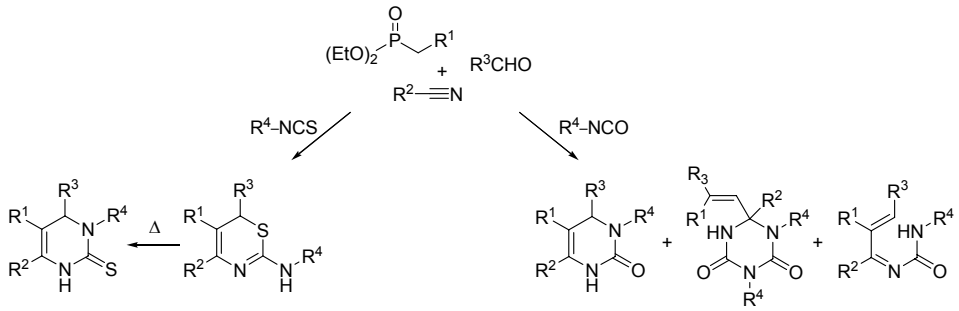
gebruik te maken van een multi-component benadering. Beide strategieën leveren een enorme flexibiliteit in de synthese van dihydropyrimidine nucleosiden op en worden uitvoerig beschreven in de hoofdstukken drie tot en met zes.



Figuur 8.1 Mureidomycin A en dihydropyrimidine nucleosides

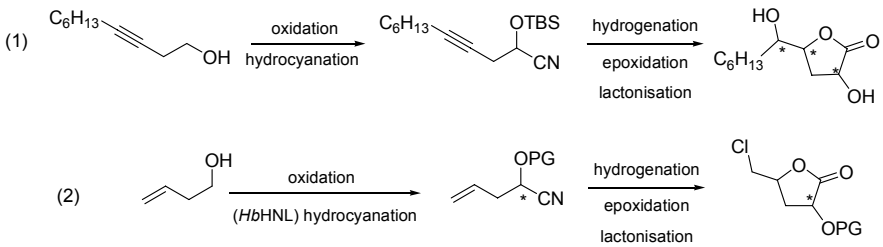
Dihydropyrimidines **C** kunnen gesynthetiseerd worden via een vier-componenten reactie (4CR) waarbij fosfonaten, nitrillen, aldehydes en isocyanaten onder basische condities met elkaar reageren. De mogelijkheden en de beperkingen van deze multicomponent reactie zijn uitgebreid beschreven in hoofdstuk 3, waarbij variatie van alle vier de componenten onderzocht is. De variatiemogelijkheid in het nitril en aldehyde is zeer uitgebreid, terwijl variatie in het fosfonaat beperkt is. Het gebruik van andere fosfonaten dan diethyl methylfosfonaat leidde in de meeste gevallen tot een omleggingsreactie waarbij fosforamidaten gevormd werden. Variatie van het isocyanaat leidde tot de vorming van verscheidene producten: dihydropyrimidines, imines en triazinane dionen (Schema 8.1). Verder resulteerde de toepassing van een chiraal aldehyde in de vorming van een dihydropyrimidine met een *de* van 80 %, hetgeen veelbelovend is voor een eerste studie met chirale startmaterialen.

Wanneer in plaats van isocyanaten, isothiocyanaten gebruikt werden in de 4CR, leidde dit niet tot de verwachte thio-dihydropyrimidines **C**, maar werden er thiazines gevormd (Schema 8.1). In een studie met vergelijkbare thiazines met een ester op de C5-positie is aangetoond dat de thiazines omgezet konden worden in thio-dihyropyrimidines **C** middels verwarming in een magnetron. De omlegging van de thiazines zonder ester functie op de C5-positie bleek reeds plaats te vinden bij 120°C, terwijl de thiazines met een ester groep dat pas bij 210°C deden.



Schema 8.1 Verschillende producten van de 4CR

Het tweede deel van de dihydropyrimidine nucleoside **A**, het 3'-deoxyribose deel **B**, kan gesynthetiseerd worden via een *de novo* chemo-enzymatische cascade reactie uitgaande van een eenvoudige alcohol. Om inzicht te krijgen in de mogelijkheden van deze snelle selectieve chemo-enzymatische reactie, is eerst een chemische model studie uitgevoerd. Daarin is begonnen met een relatief gemakkelijk te hanteren alcohol: 3-decyn-1-ol, dat omgezet is in 3'-deoxy-5'-hexyllacton in 44% opbrengst over 5 stappen ((1) in Schema 8.2).



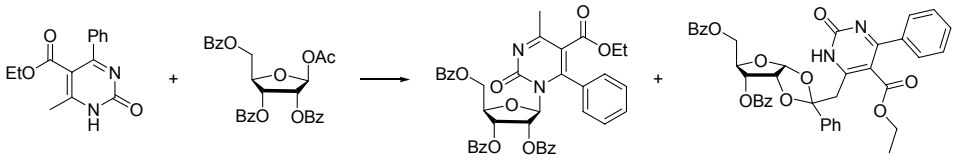
Schema 8.2 Synthese van 3'-deoxylactonen

Vervolgens is op een zelfde wijze 3-buten-1-ol, dat veel vluchtiger en isomerisatie gevoeliger is, omgezet in 3'-deoxy-5'-chloorlacton ((2) in Schema 8.2). In de laatste stap van de synthese, de door hydrolyse geinduceerde lactonisatie, is gebruik gemaakt van HCl, wat leidde tot de vorming van een 5'-chloorlacton. De invoering van een chloor atoom op de 5'-positie zorgt ervoor dat er geen verschil meer gemaakt hoeft te worden tussen 2'- en de 5'-positie, waardoor vervolg reacties gemakkelijker uitgevoerd zullen kunnen worden (Schema 8.2).

Tenslotte, is deze chemische route omgezet naar een chemo-enzymatische route, waarbij een stereocentrum op C2 geïntroduceerd wordt. Een algemene procedure is ontwikkeld voor de synthese van γ,δ-onverzadigde cyanohydrinen vanuit γ,δ-onverzadigde alcoholen waarbij een (geimmobiliseerde) TEMPO/PhI$(OAc)_2$ oxidatie is gecombineerd met een *Hevea Brasiliensis*

hydroxynitrile lyase (*Hb*HNL) gekatalyseerde hydrocyanering. Dit leidde tot hoge *ee*'s van de cyanohydrine producten.

Om tenslotte het hoofddoel van dit onderzoek te voltooien, de synthese van dihydropyrimidine nucleosiden **A**, is er een model studie uitgevoerd naar de koppeling van natuurlijke ribosen aan de in hoofdstuk 3 gesynthetiseerde dihydropyrimidines. Helaas leidden tot nut toe, noch de Vorbrüggen condensatie, noch een nucleofiele substitutie tot de vorming van de gewenste dihydropyrimidine nucleosiden. Wanneer in de Vorbrüggen condensatie echter een overeenkomstig pyrimidine gebruikt werd, leidde dit wel tot de vorming van een nucleoside (Schema 8.3). De nucleobase is echter niet verbonden via N1, maar via N3 aan de ribose. Bij het gebruik van TMSOTf als Lewis zuur werd afhankelijk van de reactietemperatuur ook bijproducten gevormd, een daarvan is een ortho-ester (Schema 8.3). Op dit moment worden verder studies uitgevoerd om wel tot de N1 gebonden dihydropyrimidine nucleosiden te komen.



Schema 8.3 Vorbruggen condensatie van pyrimidine met ribose

Uiteindelijk is er ook een begin gemaakt met de synthese van mureidomycine A. De synthese van de verschillende aminozuur bouwstenen van het peptide deel is voltooid. Het niet-symmetrisch ureum kon verkregen worden uit de trifosgeen koppeling van *m*-tyrosine met L-methionine. 2-Amino-3-methylaminobutaan zuur (AMBA) kon gesynthetiseerd worden via een 6 staps synthese waarbij gestart werd met threonine. De synthese van MRD A staat echter nog in de kinderschoenen en in de toekomst zullen de verschillende aminozuren aan elkaar gekoppeld moeten worden. De verwachting is dat dit gedaan kan worden via standaard peptide koppelingen. Tenslotte, wordt op dit moment onderzocht hoe de enamide binding in MRD A het beste gesynthetiseerd kan worden, waarmee de totaalsynthese van MRD A steeds dichterbij komt.

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Dankwoord

En dan zitten de vier jaar er al weer op! Daarover een boekje schrijven is een hele klus, maar het meest gelezen deel schrijven zonder typefouten en niemand te vergeten is ook niet gemakkelijk. Want alleen had ik dit niet allemaal kunnen doen.

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De heren van de lees- en/of promotiecommissie, prof.dr. F. Bickelhaupt, prof.dr. G. van der Marel, dr. U. Hanefeld en dr. R. Pieters, bedankt voor jullie correctie van mijn proefschrift en de aanwezigheid tijdnes de verdediging. In addition, the foreign referees, prof.dr. O. Kappe and Dr. M. Elliott thank you for reading and correcting this thesis.

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Naast de hulp “binnenshuis” zijn de verschillende samenwerkingen ook zeer vruchtbaar geweest. Lars en Ulf, bedankt voor jullie goede samenwerking. Jullie expertise hebben we uiteindelijk toegepast in de synthese van de 3’-deoxylacton. Oliver and Toma, thank you very much for the great collaboration on the thiazine-dihydropyrimidinethiones

rearrangement of chapter 4. Within a very short period a nice paper was written, although Toma and I had never met before. Carel, helaas heeft onze samenwerking tot nu toe niet tot een publicatie geleid, maar hopelijk is er in de toekomst weer mankracht om het toch nog op te pakken.

Ook de vele studenten die voorbij gekomen zijn, wil ik natuurlijk hartelijk bedanken voor hun bijdrage aan dit onderzoek. Bastian, mijn eerste student toen ik zelf nog maar drie maanden aio was. Het begeleiden heb ik door/met jou kunnen leren. Altijd in voor een gezellig praatje, borrel bezoeken en dan met name de BBB met kasteelbier… Sander, van te voren was ik voor je gewaarschuwd, omdat je tijdens het practicum niet altijd de meest handige zou zijn geweest. Hier heb ik niets van gemerkt en je hebt je bij mij in drie maanden voorbereid op je buitenlandse stage in Berlijn. Helen, jij hebt aan een zijproject, de synthese van de bouwstenen van mureidomycine A, gewerkt. In de synthese van AMBA, zoals in hoofdstuk 7 beschreven, bleek achteraf dat we de verkeerde keuzes hebben gemaakt. Gelukkig was de synthese van het niet-symmetrisch ureum succesvoller… Veel succes met je eigen promotieonderzoek! Renske, een ijverige student die wist wat ze deed! Je hebt dan ook een wezenlijke bijdrage geleverd aan de synthese van het 3'-deoxylacton zoals beschreven in hoofdstuk 6. Anass, je bleef maar terugkomen, ook al was ik vertrokken bij OAC. Twee keer heb je bij mij stage gelopen, nu bij Rachel om maar liefs twee Master diploma's te behalen. Het was altijd erg gezellig met je op het lab, want wat kan jij kletsen zeg! Michiel, drie vrouwen op een lab en een vierde erbij, dan zou toch echt een te groot kippenhok worden. En dus kwam jij bij mij stage lopen, nou toen was het kippenhok op zijn hoogtepunt! Helaas werden de oorspronkelijke drie maanden wat langer, desondanks heb je het met succes afgerond. Kanar, hoofdstuk 5 van dit proefschrift is grotendeels jouw werk. Samen cyanohydrine reacties doen, zorgde voor een hele dag keihard werken met een resultaat dat er mag wezen. Samen met Marien moest je op het eind een zuurkast delen en dat deden jullie op geheel eigen wijze. Een streep door het midden van de zuurkast met daarnaast een bufferzone! Stond er iets in de bufferzone dan werd dat wel duidelijk gemaakt aan de ander. Marien, last but not least… Je kreeg een van de moeilijkste projecten, maar aan het eind van je stage zei je dat je wel een potje met mureidomycine A op mijn bureau zou zetten. Helaas is het niet zover gekomen….

Naast studenten, waren er ook de vele collega's die mijn promotietijd erg aangenaam maakten. Robin, Bas en Mieke, samen hebben we vele keren op een borrel op de VU, in de stad of bij een van ons thuis een of meerdere drankjes gedronken. Een ontzettend leuke tijd waarvan ik erg heb genoten. Robin, altijd was het gezellig met je, en voor menig vrouw ook wel gevaarlijk… Bas met jou heb ik menig keer bij Achmed gezeten (na een borrel) om toch maar telkens weer te proberen of ze nu wel sito-sticks hadden. Mieke, een paar jaar

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Danielle

Curriculum Vitae

De auteur van dit proefschrift werd geboren op 30 augustus 1979 te Tilburg. Na het behalen van het Atheneum diploma in juni 1997 aan het Maurick College te Vught, werd begonnen met een studie Chemie aan de Hogeschool West Brabant in Etten-Leur. Van februari 2000 tot oktober 2000 werd in het kader van stage/afstuderen en doorstuderen aan de Vrije Universiteit onderzoek gedaan bij de sectie organische en anorganische chemie (prof.dr. K. Lammertsma) van de Vrije Universiteit naar de reactie van fosfinidenen met imines.

Na het afleggen van het ingenieurs examen in februari 2001 werd de studie Scheikunde voortgezet aan de Vrije Universiteit te Amsterdam. Van augustus 2001 tot januari 2002 werd tijdens een hoofdvakstage onderzoek gedaan aan de Westfälische-Wilhelms Universität Münster (prof.dr. G. Erker) op het gebied van zirkonium-fosfor chemie. Het doctoraal examen werd in mei 2002 behaald. In diezelfde maand werd gestart met het promotieonderzoek in de Bio-Organische Synthese onder leiding van dr. ir. R. V. A. Orru en later prof.dr. M. B. Groen, hetgeen heeft geresulteerd in het voor u gelegen proefschrift. Tijdens deze periode van vier jaar werden de behaalde resultaten gepresenteerd op nationale en internationale congressen in binnen- en buitenland, zoals het 2nd Multi Component Reaction conference in Genua, Italië en Biotrans in Delft, Nederland. Vanaf augustus 2006 is Danielle werkzaam als post-doc bij het radionucliden centrum en de KNO afdeling van het VUmc (prof.dr. G. A. M. S. van Dongen) in samenwerking met Philips.

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